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FROM THE OFFICE OF THE PRESIDENT

In accordance with Article IX, Section 3 of the Constitution of the International Academy of Gynecological Cytology the following scientific sub-committee of the Academy has been established:

Terminology Sub-Committee

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WHO'S WHO IN THE ACADEMY

The "Who's Who in the Academy" will publish in every issue of the ACTA CYTOLOGICA biographies of some of the Members of the International Academy of Gynecological Cytology.

INFORMATIONS BIOGRAPHIQUES

Cette rubrique publiera dans chaque issue des ACTA CYTOLOGICA des biographies sur quelques Membres de l'Académie Internationale de Cytologie Gynécologique.

BIOGRAPIEN VON MITGLIEDERN DER AKADEMIE

In diesem Abschnitt der ACTA CYTOLOGICA werden in jeder Ausgabe Biographien von Mitgliedern der Internationalen Akademie für Gynäkologische Zytologie zum Abdruck gebracht.

QUIÉN ES QUIÉN EN LA ACADEMIA

La seccion QUIÉN ES QUIÉN EN LA ACADEMIA publicara en cada número de la ACTA CYTOLOGICA biografias de algunos de los Miembros de la Academia Internacional de Citologia Ginecologica.

GEORGE N. PAPANICOLAOU
THE HONORARY PRESIDENT OF THE ACADEMY

"For one of the greatest contributions of the century to the health of women," Dr. George N. Papanicolaou, in June, became the recipient of the 1957 Century award of the General Federation

of Women's Clubs "in recognition of his development of the uterine cancer cell examination, which has helped save the lives of thousands of women, and which promises to eliminate cancer of the uterus as a major cause of death." Behind this significant award is the story of a man of imagination and faith, of humility and dedication.

Dr. Papanicolaou is the one man who, more than anyone else, has made it possible to educate today's women that they need not die of their second most deadly cancer foe—uterine cancer. His original studies in cytology have led to development of the technique, sometimes called the "Pap smear," which makes possible the detection of cancer of the uterus before it has given rise to any symptoms—at which stage it is almost 100 per cent curable.

From the beginning his restless, inquiring mind sought different horizons than those usually afforded the physician. Born in Coumi, Greece, in 1883, he had received his medical degree from the University of Athens in 1904 and his Ph.D. at Munich in 1910. In 1911 he accepted a post as physiologist in an expedition organized by the Prince of Monaco. Then came the Balkan War, and the young Dr. Papanicolaou joined the Medical Corps of the Greek Army, serving about a year.

In 1913 he and his wife Mary came to America, having now definitely decided that he wanted to do research in the medical and biologic sciences. He started out as a $60-a-month researcher in the Department of Pathology of New York Hospital. But the young Greek doctor rapidly moved on and up. About a year later he became a research associate in the Department of Obstetrics and Gynecology, Cornell University Medical College, 1916-23 Instructor; 1923-37 Assistant Professor; 1937-47 Associate Professor; 1947, Professor of Clinical Anatomy, as well as Consultant of the Kate Depew Strang Clinic at Memorial Hospital, New York Hospital, and the National Cancer Advisory Board.

Although Dr. Papanicolaou's researches have been in the fields of anatomy, pathology and endocrinology, with special attention to the physiology of reproduction, it is the development of the uterine cancer cell examination which places him in the company of such great men as Semmelweis, Pasteur, Jenner, and others. Early in the century, the president of the American Gynecological Society had gloomily announced that uterine cancer was incurable. Today, Dr. Charles S. Cameron, former Medical and Scientific Director of the American Cancer Society, says, "We have reached the point where we believe that if the uterine cancer cell examination could be given to all adult women, loss of life from uterine cancer could be almost totally eliminated."

The story goes back to 1917 when Dr. Papanicolaou with the late Professor Charles B. Stockard, in the course of an investigation totally unrelated to the problem of cancer, discovered the value of a vaginal smear as a method of determining the sequence of certain changes in the reproductive organs of the female guinea pig. But it was not until 1923 that the first use was made of this vaginal smear in diagnosis of cancer of the uterus. In that year, Dr. Papanicolaou began a comprehensive study of human vaginal fluids at the Women's Hospital in New York City, and because he felt that the exploration should not be limited to the examination of smears from normal women alone, it was extended to patients with different pathologic conditions, including cancer.

As more cases were examined and more diagnoses confirmed, the practical usefulness and reliability of the vaginal smear as a detection technique became obvious, and Dr. Papanicolaou reported these early studies in a brief preliminary paper in 1928, "The first observation of cancer cells in a smear of the uterine cervix," he said, "gave me one of the greatest thrills I ever experienced during my scientific career." Later he felt constrained to add, "I failed to create much faith among my colleagues in the practicability of this procedure. The prevailing opinion, as expressed to me by one of the most outstanding pathologists of that time was that 'since the uterine cervix was accessible to diagnostic exploration by biopsy, which is a relatively simple procedure, the use of a cytologic examination is superfluous.'" Indeed most of the outstanding doctors and pathologists of that time concurred in this opinion. The consensus was that the cytologic examination of vaginal smears was important only for the purpose of research.

Certainly this must have been a difficult and discouraging time. For the next ten years he devoted his research toward other possible applications of the cytologic method—including studies of the action of sex hormones upon the accessory organs of reproduction and its evaluation by cytologic criteria. This work was conducted in cooperation with Dr. Ephraim Shorr of the Department of Medicine of Cornell University Medical College. It resulted in the recognition of certain cytologic patterns in the vaginal smears which proved to be very useful for evaluating the effect of some hormones and of their therapeutic action.

In 1939, in association with Dr. Herbert F. Traut and later with Dr. Andrew A. Marchetti, experienced gynecologic pathologists, Dr. Papanicolaou resumed the study of vaginal smears and their application in cancer diagnosis at Cornell Medical College and the Women's Clinic of the New York Hospital. The results of these studies were published in 1943 and were soon confirmed and endorsed by members of the staffs of Harvard Medical School, the Vincent Memorial Laboratories in Boston, and the New York Post-Graduate Medical School. This was the turning point in the attitude of the medical profession toward the use of the vaginal smear, or the uterine cancer cell examination, as it is now known, as a diagnostic approach to detection of cancer of the uterus and cervix.

Dr. Papanicolaou, speaking of those early days, says that what happened "may be compared to an avalanche, which once started rolling, constantly kept gathering speed and more strength, imparted to it by the many contributions of gifted investigators who devoted their talents to this field."

In 1948 the American Cancer Society sponsored the first National Cytology Conference, out of which came the statement that of all cancer detection procedures available, this approach (the uterine cancer cell examination) was unique in that the method detects early cancer before it is visible to the naked eye and before it can produce the danger signals of cancer. A whole new vista of hope was unfolded

for women. In 1954, the scientific session at the American Cancer Society's Annual Meeting dealt with the potentialities of exfoliative cytology, and the press of the nation carried many articles concerning the papers presented. The public was becoming aware of the work of Dr. Papanicolaou. Earlier, pilot projects for mass cytologic screenings had been initiated in order that an epidemiological study might be made as to the effectiveness of the uterine cancer cell examination.

At the Third National Cancer Conference held in Detroit in June 1956, and attended by scientific men and physicians from all over the world, the results of some of these studies were reviewed and consensus was "that cytology is the most sensitive and exact method available for the detection of early cervical cancer." There is now widespread acceptance of the Papanicolaou method as a great life-saving detection tool and it appears realistic to anticipate the day when wider use of the uterine cancer cell examination technique may make it possible to virtually eliminate cancer of the cervix as a cause of death.

Ever the careful man of science, Dr. Papanicolaou stresses the limitations of the examination. He says,"In spite of great advances made in the cytologic diagnosis of cancer, we still have to depend on the biopsy as the most conclusive proof of cancer. A positive smear report is not a final diagnosis in itself. It does not eliminate the need for a careful clinical work-up and a thorough examination of a tissue section by a qualified pathologist." Dr. Papanicolaou is credited with being responsible for the training of most of the leading cytologists in this country and abroad. Indeed, one of his disciples is his own very attractive young niece, who is studying management of cancer clinics so that she can introduce new methods in Greece.

The honor bestowed on Dr. Papanicolaou by the General Federation of Women's Clubs is but the latest of many that he has earned. In 1948 he received the Borden Award of the Association of American Medical Colleges and the Amory Award of the American Association of Arts and Sciences; in 1950 the Lasker Award of the American Public Health Association; 1951 the First Award of the Order of AHEPA, as the most outstanding American scientist of Greek descent; in 1952 the American Cancer Society Award for Distinguished Service to Cancer Control; in 1953 the Wien Award and the Royal Order of Phoenix; in 1954 the Modern Medicine Award; in 1955 the Bertner Foundation Award, and there were others.

Yet despite all these honors, Dr. Papanicolaou remains the earnest, dedicated researcher. Writing in a scientific journal, he says, ". . . one should not lose sight of the fact that the cytological method which has met with universal favor within the past few years because of its successful application in cancer detection and diagnosis, is an outgrowth of fundamental biological studies. In this we may find the elements of another noteworthy example of the all-important role of basic research in opening new and ever-widening avenues of investigation leading to accomplishments of broad significance for the advancement of science and the benefit of mankind."

What is Dr. Papanicolaou doing today? He travels daily from his home in Douglaston, Long Island, to his office at Cornell University Medical College in New York City. Usually Mrs. Papanicolaou does the driving. She assists him in the office, keeping records and managing the detail work. The American Cancer Society has arranged for an annual grant of $10,000 to Cornell Medical College, which along with additional support from Cornell and the Albert and Mary Lasker Foundation, will enable Dr. "Pap" now Emeritus Professor of Clinical Anatomy, to concentrate all his time and energy on cancer research.

Dr. Papanicolaou is held in particular esteem by his associates and students for his rare faculty of sharing with others the skill and knowledge he has developed over the years, as well as for his unfailing kindliness and sympathy. Never has he lost his hunger for and appreciation of beauty, nor the reverence and awe that filled him as a young boy for the manifestations and forces of creative nature. Even in his most learned papers he pays them tribute. At the presentation of the Passano Award in Chicago in June 1956 he said, "One cannot help but admire the wonderfully balanced mechanism of this ceaseless process of death and regeneration, which actually constitutes a continuous rejuvenation of the body and forms the physical basis of our every-renewing personality."

His recreations are simple—swimming, a little gardening, long walks. Sometimes, accompanied by Mrs. Papanicolaou at the piano, he'll take up the violin, and together they'll enjoy the music of their youth. He may not know George Chapman's verse, but most certainly these lines typify him who has made "one of the greatest contributions of the century to the health of women."

"So our lives
In acts exemplary, not only win
Ourselves good names, but doth
to others give
Matters for virtuous deeds, by
which we live."

By Esther Allegretti, from "Cancer News," Summer 1957.

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133. 1957. Cellular Changes in the Development of Pulmonary Cancer as Revealed by Cytology: A Case Report. (in press)

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THE SYMPOSIA BY CORRESPONDENCE OF ACTA CYTOLOGICA

INTRODUCTORY REMARKS

The Symposia of ACTA CYTOLOGICA are *held entirely by correspondence* and contain international discussions of scientific problems of interest to the exfoliative cytologist.

System for Selecting Subjects for Symposia: From recommendations received, the Editorial Office will draw up the list of subjects and will publish these subjects in ACTA CYTOLOGICA, under the heading FUTURE SYMPOSIA.

The final detailed program will be published in ACTA CYTOLOGICA immediately preceding the one where these topics are to be considered, under the heading, THE NEXT SYMPOSIUM.

Instructions for Authors: Each problem will be introduced by a *Main Speaker* or *Speakers*. These principal papers will then be considered by persons identified as *Discussants*. As a general rule, approximately 600 words each will be allocated for main papers and 200 words each will be allocated for the contributions of the Discussants. The Main Speakers will then be given the opportunity to make unlimited Closing Remarks.

Photomicrographs and tables may be reproduced: one full page for each principal paper and for the paper of the Discussant (maximum one-half page per contribution). The photomicrographs and tables should be submitted in glossy photographic prints, preferably in the size of 3×4 inches (i.e., 12×19 cm) and should show a proportional 10μ scale on its reverse side. *Each figure should be accompanied by a comprehensive caption.*

The Discussants are requested *to strictly restrict their contributions to the discussion of the main papers.* Discussions which are not directly related to the main paper cannot be accepted. It is suggested that the Discussants prepare their contributions in such a manner that the reader may gain the impression of an actual round table conference.

The Closing Remarks of the Main Speakers should be limited to the answering of questions raised in the discussion and to other directly related information.

The Bibliography for the papers of both Main Speaker and Discussant should be organized in the same manner as in the American Journal of Obstetrics and Gynecology, at the *end* of the paper. *Every cited opinion or publication should have a reference in the bibliography.*

Deadline for Contributions: The Editorial Office will set deadlines for each written symposium. These will include:

1. deadline for agreements to contribute.
2. deadline for main papers.
3. deadline for discussions.
4. deadline for closing remarks.

Reprints: Authors may receive reprints of their papers by ordering these reprints before the particular issue goes to press. There will be a nominal charge for reprints: $6.00 per page for the first one hundred copies, and $3.00 per page for each additional hundred.

LES SYMPOSIA PAR CORRESPONDANCE DES ACTA CYTOLOGICA

Les *Symposia par Correspondance* des ACTA CYTOLOGICA présentent des discussions internationales sur des problèmes scientifiques intéressant le cytologiste exfoliative.

Système du choix des sujets pour les symposia: En partant des propositions, et sous la rubrique: FUTURS SYMPOSIA, le bureau de rédaction dressera la liste des sujets principaux qui seront publiés dans les ACTA CYTOLOGICA.

Le bureau de rédaction établira le programme définitif et détaillé des discussions qui sera publié dans les ACTA CYTOLOGICA précédant immédiatement le symposium, sous la rubrique PROCHAIN SYMPOSIUM.

Recommandations pour les auteurs: Chaque sujet principal sera présenté par un Rapporteur Général ou des Rapporteurs. Ces mémoires principaux seront alors soumis aux Participants à la Discussion. En règle générale 600 mots seront accordés aux Rapporteurs des sujets principaux, et, 200 mots aux Participants à la Discussion. Les Rapporteurs Généraux pourront clôturer les discussions par un nombre illimité de remarques.

Des microphotos et graphiques pourront être reproduits à raison d'une page entière pour chaque sujet principal et une demi page au maximum pour les discussions. Les microphotos et les graphiques doivent être présentés sur du papier brillant, de préference dans le format 12 × 19 cm. *Chaque figure devra être accompagnée d'une legende explicative précise.*

Les membres et invités prenant part aux discussions sont invités à *limiter strictement leurs interventions aux discussions des sujets principaux.* Des discussions qui n'ont pas de rapport direct avec le sujet principal *ne pourront être acceptées.* Il est recommandé que les discussions soient rédigées d'une manière telle que le lecteure ait l'impression d'assister à une discution réelle de table ronde.

Les Remarques de Clôture du Rapporteur Général devront se limiter à la réponse aux questions soulevées dans les discussions et aux autres informations éventuelles ayant un rapport direct avec le sujet.

La bibliographie des rapports et discussions devra être rédigée de la même manière que celle de l'American Journal of Obstetrics & Gynecology et figurer à *la fin* du texte. *Chaque opinion ou publication citée dans le texte doit avoir sa réference dans la bibliographie.*

Dates limite pour les collaborations: Le bureau de rédaction, fixera des dates limites comprenaet:

1. un délai pour l'acceptation des collaborations,
2. un délai pour les sujets principaux,
3. un délai pour les discussions,
4. un délai pour les remarques de clôture.

Tirés-à-part: les auteurs pourront obtenir des tirés-à-part de leurs communications en les demandant avant la mise sous presse des ACTA CYTOLOGICA publiant leurs articles. Les tirés-à-part seront facturés: $6.00 par page de texte pour le premier cent et $3.00 pour chaque centaine supplémentaire.

DIE SCHRIFTLICHEN SYMPOSIEN DER ACTA CYTOLOGICA

Die schriftlichen Symposien der ACTA CYTOLOGICA befassen sich auf internationaler Basis mit wissenschaftlichen Problemen, die für den Exfoliativ-Zytologen von Interesse sind.

System der Thema-Auswahl für die Symposien: Die Schriftleitung stellt auf Grund von Thema-Vorschlägen eine Liste von Haupt-Themen zusammen, und gibt diese Liste unter dem Titel ZUKÜNFTIGE SYMPOSIEN bekannt.

Die Schriftleitung bereitet das Programm mit allen Einzelpunkten vor, und veröffentlicht dieses Programm in dem Heft, das dem betreffenden Symposion vorausgeht, unter dem Titel DAS NÄCHSTE SYMPOSION.

Instruktionen fur Autoren: Jedes Thema wird von einem oder mehreren Referenten behandelt. Diese Referate werden dann von Diskussions-Vortragenden besprochen. Im allgemeinen werden Referate auf etwa 600 Worte beschränkt, und Diskussions-Vorträge auf 200 Worte. Die Referenten erhalten dann die Gelegenheit, Schlussbemerkungen ohne Wortzahlbeschränkung zu machen.

Mikrophotographien und Tabellen können abgedruckt werden: eine Ganzseite kann Referenten und eine halbe Seite Diskussionsvortragenden für Abbildungen zur Verfügung gestellt werden. Die Photographien sind auf Hochglanzpapier, und möglichst in der Grösse 12 × 19 cm erbeten und soll ein proportionales 10μ Zeichen auf der Rückseite haben. *Jede Abbildung muss von einem erklärenden Untertitel begleitet sein.*

Die Diskussionsvortragenden sind gebeten, sich in ihren Beiträgen *streng an das Hauptreferat zu halten.* Diskussionsbeiträge, die sich nicht an das Hauptthema halten, *können nicht berücksichtigt werden.* Es wird vorgeschlagen, dass die Diskussionsvorträge in einem Stil abgefasst sind, dass der Leser den Eindruck gewinnt, als ob es sich um eine Diskussion am runden Tisch gehandelt hätte.

Die Schlussbemerkungen der Referenten sollen sich nach Möglichkeit auf die Beantwortung von Diskussionsfragen beschränken.

Die Bibliographie der Referate und der Diskussions-Vortäge sollen *am Schluss* der Beiträge nach dem Muster der Bibliographien im American Journal of Obstetrics and Gynecology aufgeführt werden. *Jede zitierte Ansicht oder Publikation muss eine Referenz in der Bibliographie haben.*

Termine für Beiträge: Die Schriftleitung setzt Termine für die Schriftlichen Symposien fest. Die folgenden Termine werden bekanntgegeben:

1. Termin für Erhalt der Beitrags-Zusagen,
2. Termin für Erhalt der Hauptreferate,
3. Termin für Erhalt der Diskussions-beiträge.
4. Termin für Erhalt der Schlussbemerkungen.

Sonderdrucke: Autoren können Sonderdrucke ihrer Beiträge bestellen, bevor die betreffende Ausgabe in Druck geht. Die Schriftleitung muss diese Sonderdrucke berechnen und wird einen Betrag von $6.00 pro Seite und 100 Sonderdrucke, und einen Betrag von $3.00 für jedes weitere Hundert erheben müssen.

SIMPOSIUM ESCRITO DE ACTA CYTOLOGICA

El simposium escrito de ACTA CYTOLOGICA contiene discusiones internacionales sobre problemas científicos que son de interés para el citólogo exfoliativo.

Sistema de selección de materias para el simposium: Con sugestiones recibidas, la oficina editorial confeccionará una lista de los temas más interesantes, lista que será publicada en ACTA CYTOLOGICA con dos numeros de anticipación a la fecha de su posible publicación, bajo el epígrafe de "SIMPOSIUM FUTUROS."

La Oficina Editorial confeccionará y publicará una lista detallada del programa de la discusión en el número de ACTA CYTOLOGICA immediatamente anterior a aquel en que han de ser incluidos los temas, bajo el epígrafe de: EL PROXIMO SIMPOSIUM.

Participación en el Simposium Escrito: No habrá restricción alguna sobre el número de puntos de discusión en los que cualquier autor desee participar.

Instrucciones a los Autores: Cada problema deberá ser presentado por un ponente o ponentes. Estos trabajos principales serán entonces discutidos por los comunicantes. Como regla general, se permite un máximo de 600 palabras para los trabajos principales y 200 palabras para las contribuciones de los comunicantes. Al ponente principal se le da la oportunidad de hacer rectificaciones finales ilimitadas.

Pueden reproducirse microfotografías y tablas: una página por cada trabajo principal y un máximo de media página por discusion. Las microfotografías y tablas deberán enviarse en forma de copias fotográficas amplias. A ser posible de 3 × 4 pulgadas (12 × 19 cms). *Cada figura deberá acompañarse de su correspondiente leyenda.*

Se suplica a los comunicantes *ajustar estrictamente sus comunicaciones a la discusión de los trabajos principales.* Las discusiones que no estén directamente relacionadas con el trabajo principal *no podrán ser aceptadas.* Se sugiere que los comunicantes realicen sus contribuciones de manera tal que el lector tenga la impresión de estar ante una verdadera mesa redonda.

Las rectificaciones finales de los ponentes deberán limitarse a contestar las preguntas aparecidas a lo largo de la discusión asi como a otras directamente relacionadas con el tema.

La bibliografia, tanto de las ponencias como de las comunicaciones deberá redactarse de la misma forma que figura en el American Journal of Obstetrics and Gynecology, *al final* del trabajo. *Toda opinión o publicación citada deberá tener su correspondiente referencia en la bibliografía.*

Fechas para las contribuciones: La Oficina Editorial, fijará fechas límite absolutas para cada simposium escrito. Estas incluirán:

1°. Fecha límite para acuerdo de contribución,
2°. Fecha límite para las ponencias,
3°. Fecha límite para las discusiones,
4°. Fecha límite para las anotaciones finales.

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Article I, Section 16 of the Bylaws of the International Academy of Gynecological Cytology:
"In the Circular Letter of the Academy, no title shall be used in reference to Members of the Academy regardless of whether or not they hold a professorial rank, and regardless of which type of doctoral degree they hold, except for those cases where a further specification is necessary . . . ."

(The participating Non-Members are identified with their addresses and titles in footnotes within the symposium)

PROGRAM OF THE WRITTEN SYMPOSIA OF THIS ISSUE

I. Subject on Cancer Cytology

DEFINITION, MORPHOLOGY, CYTOCHEMISTRY AND DIAGNOSTIC IMPORTANCE OF DYSKARYOTIC CELLS

(1) Introduction:
GEORGE N. PAPANICOLAOU, New York, New York, U.S.A.

(2) Definition:
RUTH M. GRAHAM, Buffalo, New York, U.S.A.
Disc.: Jean Berger, Basel, Switzerland
Leopold G. Koss, New York, New York, U.S.A.
James W. Reagan, Cleveland, Ohio, U.S.A.
Jose R. del Sol, Madrid, Spain
Peter Stoll, Heidelberg, Germany
Guillermo Terzano, Buenos Aires, Argentina

(3) Morphology:
JAMES W. REAGAN, Cleveland, Ohio, U.S.A.
GUILLERMO TERZANO, Buenos Aires, Argentina
Disc.: J. Ernest Ayre, Miami, Florida, U.S.A.

(4) Cytochemistry of Dyskaryotic Cells:
H. WERNER BOSCHANN, Berlin, Germany
Disc.: Robert C. Mellors, New York, New York, U.S.A.

(5) Ultraviolet Microscopic Observations on Dyskaryotic Cells:
GEORGE L. WIED, Chicago, Illinois, U.S.A.
Disc.: Robert C. Mellors, New York, New York, U.S.A.
Jean Berger, Basel, Switzerland

(6) Dyskaryotic Cells under the Colpomicroscope:
TASSILO ANTOINE, K. BRANDL, V. GRUENBERGER, E. KOFLER, and H. KREMER, Vienna, Austria
Disc.: George L. Wied, Chicago, Illinois, U.S.A.

(7) Unfixed Dyskaryotic Cells under the Phasemicroscope:
HANS KLAUS ZINSER, Cologne, Germany
Disc.: Peter Stoll, Heidelberg, Germany

(8) Occurrence of Dyskaryotic Cells in Trichomonas Infestation:
JEAN DE BRUX and HENRIETTE WENNER-MANGEN, Paris, France
CLARICE DO AMARAL FERREIRA, Rio de Janeiro, Brazil
JULIETA CALDERON DE LAGUNA, Mexico, D.F., Mexico
Disc.: J. Ernest Ayre, Miami, Florida, U.S.A.
John F. Fiorino, Everett, Washington, U.S.A.
Claude Gompel, Brussels, Belgium
Leopold G. Koss, New York, New York, U.S.A.

(9) Dysplasia, and Occurrence of Dyskaryotic Cells in Dysplasia:
JAMES W. REAGAN, Cleveland, Ohio, U.S.A.
RUTH M. GRAHAM, Buffalo, New York, U.S.A.
Disc.: Jean Berger, Basel, Switzerland
Jean de Brux, Paris, France
Leopold G. Koss, New York, New York, U.S.A.
Edmund Schueller, Vienna, Austria
Guillermo Terzano, Buenos Aires, Argentina

(10) Occurrence of Dyskaryotic Cells in Carcinoma in Situ:
ANTHONY FRANCIS ANDERSON, Edinburgh, Scotland, Great Britain
Disc.: J. Ernest Ayre, Miami, Florida, U.S.A.
Jean Berger, Basel, Switzerland
Jean de Brux, Paris, France
W. Kenneth Cuyler, Durham, North Carolina, U.S.A.
Ruth M. Graham, Buffalo, New York, U.S.A.
Leopold G. Koss, New York, New York, U.S.A.
Edmund Schueller, Vienna, Austria
Jose R. del Sol, Madrid, Spain
Peter Stoll, Heidelberg, Germany
Guillermo Terzano, Buenos Aires, Argentina
H. K. Zinser, Cologne, Germany

(11) Occurrence of Dyskaryotic Cells in Invasive Carcinoma of the Uterine Cervix:
HANS KLAUS ZINSER, Cologne, Germany
Disc.: J. Ernest Ayre, Miami, Florida, U.S.A.
Jean Berger, Basel, Switzerland

W. Kenneth Cuyler, Durham, North Carolina, U.S.A.
John J. Sullivan, Auckland, New Zealand
Guillermo Terzano, Buenos Aires, Argentina

(12) Occurrence of Dyskaryotic Cells as a Result of Irradiation:
RUTH M. GRAHAM, Buffalo, New York, U.S.A.
Disc.: Jean Berger, Basel, Switzerland
Olle Kjellgren, Goteborg, Sweden
Guillermo Terzano, Buenos Aires, Argentina

(13) What is the Pre-Cancer Cell Complex as Compared with the Above Defined Dyskaryosis?
J. ERNEST AYRE, Miami, Florida, U.S.A.
Disc.: Jean Berger, Basel Switzerland
W. Kenneth Cuyler, Durham, North Carolina, U.S.A.
Ruth M. Graham, Buffalo, New York, U.S.A.
Leopold G. Koss, New York, New York, U.S.A.
J. C. de Laguna, Mexico, D.F., Mexico
Herbert E. Nieburgs, New York, New York, U.S.A.
M. June Scudamore, London, England
Jose R. del Sol, Madrid, Spain
John J. Sullivan, Auckland, New Zealand
Guillermo Terzano, Buenos Aires, Argentina
H. K. Zinser, Cologne, Germany

(14) Dyskaryotic Cells in Pernicious Anemia:
RUTH M. GRAHAM, Buffalo, New York, U.S.A.
Disc.: Cyrus E. Rubin, Seattle, Washington, U.S.A.

(15) Statistical Data on Dyskaryotic Cells:
GUILLERMO TERZANO, Buenos Aires, Argentina
PETER STOLL, Heidelberg, Germany

II. Subject on Cytological Techniques

EXPERIENCES WITH VARIOUS METHODS OF FIXATION OF SMEARS

(1) Do Cellular Changes Occur as a Result of Air Drying of Smears?
J. PAUL PUNDEL, Luxembourg, Luxembourg
CLARICE DO AMARAL FERREIRA, Rio de Janeiro, Brazil
HANNAH PETERS, Bombay, India
Disc.: Jean de Brux, Paris, France
J. Ferin, Louvain, Belgium
H. E. Nieburgs, New York, New York, U.S.A.
Luis Montalvo Ruiz, Madrid, Spain
Edmund Schueller, Vienna, Austria
Peter Stoll, Heidelberg, Germany
Guillermo Terzano, Buenos Aires, Argentina

(2) Advantages and Disadvantages of Fixation Methods Other Than Alcohol-Ether:
RUTH M. GRAHAM, Buffalo, New York, U.S.A.
PETER STOLL, Heidelberg, Germany
J. PAUL PUNDEL, Luxembourg, Luxembourg
Disc.: J. C. de Laguna, Mexico, D.F., Mexico
L. Montalvo Ruiz, Madrid, Spain
G. Terzano, Buenos Aires, Argentina

(3) Fixation Techniques for Staining Procedures Other Than the Routine Papanicolaou Procedure:
CLAUDE GOMPEL, Brussels, Belgium
LUIS MONTALVO RUIZ, Madrid, Spain

III. Subject on Hormonal Cytology

ANDROGENIC EFFECT ON VAGINAL EPITHELIAL CELLS

(1) The Physiological Production of Androgens in the Normal Woman Which May Influence the Proliferation of the Vaginal Epithelium.
JOSE BOTELLA LLUSIA, Madrid, Spain
Disc.: Robert Wenner, Basel, Switzerland

(2) Is there Evidence that Androgens may be Metabolized to Substances which may have Estrogenic Effect on the Vaginal Epithelium?
ERNEST JURGEN PLOTZ, Chicago, Illinois, U.S.A.
Disc.: John A. Finkbeiner, New York, New York, U.S.A.
Karl Junkmann, Berlin, Germany
J. C. de Laguna, Mexico, D.F., Mexico
J. Paul Pundel, Luxembourg, Luxembourg
A. E. Rakoff, Philadelphia, Pennsylvania, U.S.A.

R. Wenner, Basel, Switzerland
Jose R. del Sol, Madrid, Spain

(3) Effect of Physiological Sex Hormones in Patients with Inactive Ovaries:
GEORGE L. WIED, Chicago, Illinois, U.S.A.
PETER STOLL, Heidelberg, Germany
JEAN BERGER, Basel, Switzerland
Disc.: H. W. Boschann, Berlin, Germany
J. Ferin, Louvain, Belgium
J. A. Finkbeiner, New York, New York, U.S.A.
E. J. Plotz, Chicago, Illinois, U.S.A.
G. Terzano, Buenos Aires, Argentina

(4) Is There a Physiological Cell Type Which May be Defined as "Androgenic Cell Type?"
J. PAUL PUNDEL, Luxembourg, Luxembourg
CLAUDE GOMPEL, Bruxelles, Belgium
Disc.: J. A. Finkbeiner, New York, New York, U.S.A.
H. Rauscher, Vienna, Austria
G. Terzano, Buenos Aires, Argentina

(5) Effect of Administered Androgens in Patients with Atrophic Menopausal Cell Type:
H. WERNER BOSCHANN, Berlin, Germany
Disc.: J. A. Finkbeiner, New York, New York, U.S.A.
J. Paul Pundel, Luxembourg, Luxembourg
A. E. Rakoff, Philadelphia, Pennsylvania, U.S.A.
G. Terzano, Buenos Aires, Argentina
R. Wenner, Basel, Switzerland

(6) Effect of Administered Androgens in Patients with Non-Atrophic Menopausal Cell Type:
GEORGE L. WIED, Chicago, Illinois, U.S.A.
Disc.: J. Paul Pundel, Luxembourg, Luxembourg
Guillermo Terzano, Buenos Aires, Argentina

(7) Effect of Administered Androgens in Normally Menstruating Women:
A. E. RAKOFF, Philadelphia, Pennsylvania, U.S.A.
Disc.: J. Ferin, Louvain, Belgium
J. Paul Pundel, Luxembourg, Luxembourg
Guillermo Terzano, Buenos Aires, Argentina

(8) Can one tell by means of Exfoliative Cytology an "Androgenic Cell Type" from Cell Types which occur during the Luteal Phase of the Normal Menstrual Cycle, from Cell Types which occur during Normal Pregnancy, from the "Crowded Menopausal Cell Type" and finally from the Cell Types which occur as a result of Administration of Low Dosages of Estrogens? If Yes, How?

J. PAUL PUNDEL, Luxembourg, Luxembourg
Disc.: Peter Stoll, Heidelberg, Germany
Guillermo Terzano, Buenos Aires, Argentina

(9) If Androgens Induce Growth of the Atrophic Vaginal Epithelium, is it then Correct to Refer to Degrees of Proliferation of the Vaginal Epithelium in Terms of Gradation of Estrogen Activity, such as "Slight Estrogen Effect" or "Moderate Estrogen Deficiency?"

ERICA WACHTEL, London, England, Great Britain
Disc.: J. A. Finkbeiner, New York, New York, U.S.A.
J. Paul Pundel, Luxembourg, Luxembourg
A. E. Rakoff, Philadelphia, Pennsylvania, U.S.A.
Peter Stoll, Heidelberg, Germany
Guillermo Terzano, Buenos Aires, Argentina
R. Wenner, Basel, Switzerland

(10) Threshold Dosages of Various Androgens, Using the Various Methods of Administration Necessary to Stimulate Growth of the Atrophic Menopausal Epithelium of the Vagina:

H. WERNER BOSCHANN, Berlin, Germany
Disc.: J. Paul Pundel, Luxembourg, Luxembourg

(11) Can one Determine by Means of Exfoliative Cytology the Efficiency and Duration of Androgenic Therapy?
H. WERNER BOSCHANN, Berlin, Germany
GEORGE L. WIED and ALICE M. DARGAN, Chicago, Illinois, U.S.A.
Disc.: J. Paul Pundel, Luxembourg, Luxembourg
A. E. Rakoff, Philadelphia, Pennsylvania, U.S.A.
Guillermo Terzano, Buenos Aires, Argentina

(12) Hirsutism and Vaginal Cytology:
GUILLERMO TERZANO, Buenos Aires, Argentina
ERICA WACHTEL, London, England
Disc.: Jean Berger, Basel, Switzerland
J. Paul Pundel, Luxembourg, Luxembourg

Symposium A

DEFINITION, MORPHOLOGY, CYTOCHEMISTRY AND DIAGNOSTIC IMPORTANCE OF DYSKARYOTIC CELLS

INTRODUCTION TO SYMPOSION ON DYSKARYOSIS

GEORGE N. PAPANICOLAOU
New York, New York, U.S.A.

The term "dyskaryosis" has been introduced to designate certain cytologic patterns observed in vaginal and cervical smears from cases of early carcinoma and some other pathologic lesions of the uterine cervix, in which the exfoliated cells are characterized by marked nuclear abnormalities consistent with the generally accepted cytologic criteria of malignancy, although the cells as a whole may show no significant deviation from their standard normal type.

Cells exfoliated from the normal epithelium of the vagina and the ecto- and endocervix can be grouped in four main types: the superficial, intermediate and parabasal squamous, and the endocervical. It is thus possible to recognize four distinct varieties of dyskaryosis corresponding to the four types of the exfoliated normal cells.

Such abnormal smear patterns are usually seen in cases diagnosed pathologically most frequently as intra-epithelial carcinomas of the cervix, but may also be observed in cases of cervical lesions diagnosed as non-malignant, or only potentially malignant, such as dysplasia, epidermization, and basal cell hyperactivity. Since we are still in lack of objective morphologic criteria by which the malignant nature of disputable, chiefly early lesions of the uterine cervix can be definitely established in any given case, it appears that the use of the term "dyskaryosis" for describing the above specified abnormal cytologic patterns, is most desirable. By designating and reporting such smear findings as cancerous or pre-cancerous one runs the risk of a rather subjective and, in some instances, possibly wrong conclusion, which in the presence of a clinical suspicion of a malignant neoplasm may hasten a decision in favor of a major operative procedure prior to a complete diagnostic exploration. (See figures, page 24)

THE DEFINITION OF A DYSKARYOTIC CELL

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

The term "dyskaryotic" implies an abnormal nucleus. But what kind of abnormality? Increase in size, in chromatin material? We consider that a dyskaryotic nucleus is a nucleus with all the characteristics of malignancy. First, it must have abnormal chromatin distribution. Second, it should have an increase in chromatin. Why then is this cell with a malignant nucleus not considered a true cancer cell? It is classified as a suspicious cell rather than a malignant one because too much cytoplasm is present. Occasionally it is difficult to decide whether or not there is an abnormal nuclear cytoplasmic ratio. For the cell which is difficult to classify we have an arbitrary standard. If the distance from the border of the cell is greater than the maximum diameter of the nucleus it is considered a dyskaryotic cell. If this distance is less than the masimum diameter of the nucleus the cell is classified as a differentiated squamous cancer cell.

Definition: A dyskaryotic cell is a squamous cell containing a typical malignant nucleus. It differs from a true cancer cell in having adequate cytoplasm and a cytoplasm-nuclear ratio within normal limits. (See figures, page 25)

DISCUSSION:

JEAN BERGER, Basel, Switzerland:

For the identification of the various types of cells we use a scheme (see figure, page 26) modified from that of Stoll (1). We check the cells within this scheme which are found in the smears and describe the individual cellular criteria separately on the reverse side of the sheet. Naturally, one will often find cell types which are not represented in the scheme. However, as a rule the cells which we identify as "dyskaryotic" are represented in column c, and some are in column d. These dyskaryotic cells exhibit either increased basophilic or acidophilic cytoplasm: the latter are from the cornification zone. We classify these dyskaryotic cells as Class III after Papanicolaou (suspicious).

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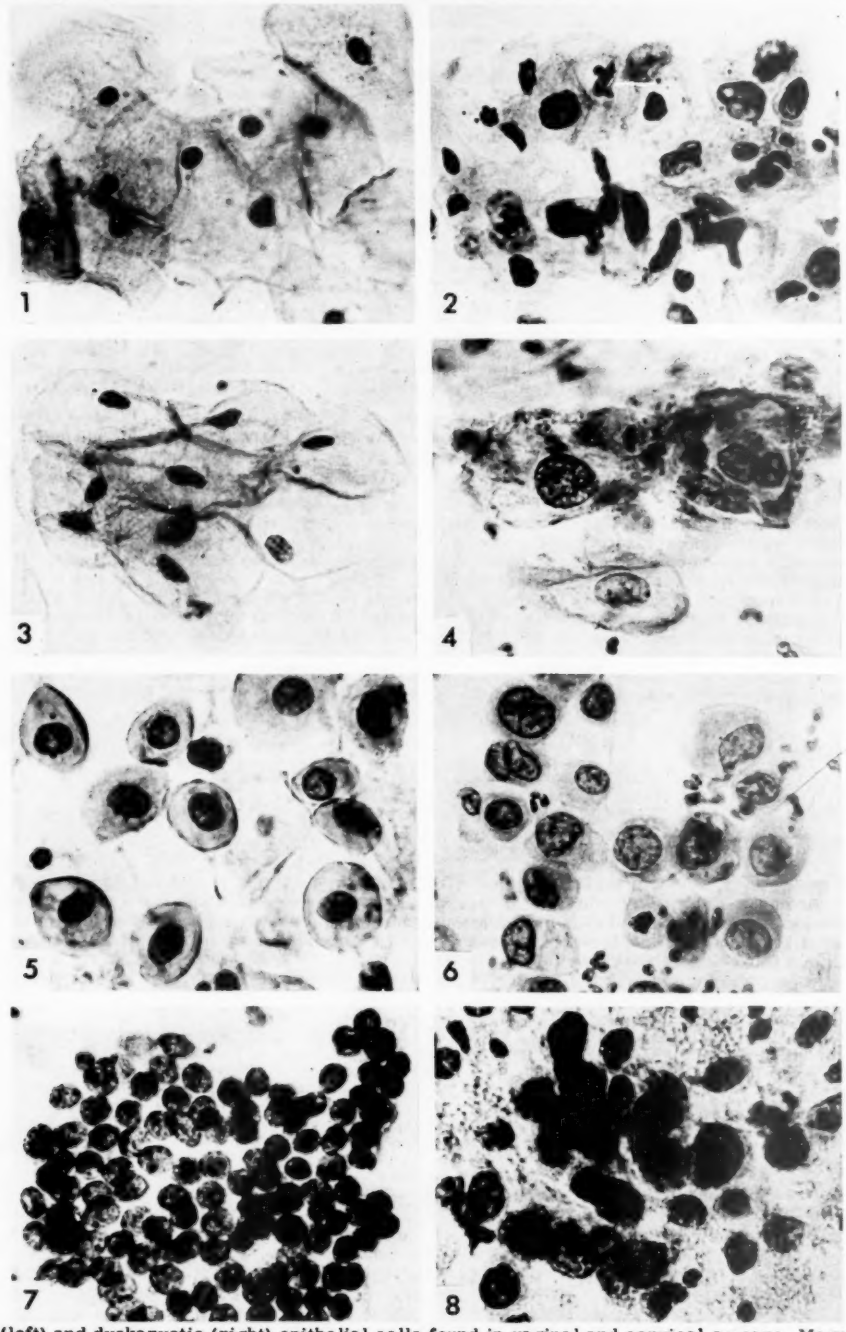
The four dyskaryotic patterns appear in smears singly or intermixed and may be recognized by clean-cut cytologic criteria as shown in the photomicrographs below.



Normal (left) and dyskaryotic (right) epithelial cells found in vaginal and cervical smears. Magnification x 600. (George N. Papanicolaou)

Figs. 1 & 2. Superficial squamous cell type.
Figs. 3 & 4. Intermediate squamous cell type.
Figs. 5 & 6. Parabasal squamous cell type.
Figs. 7 & 8. Endocervical cell type.

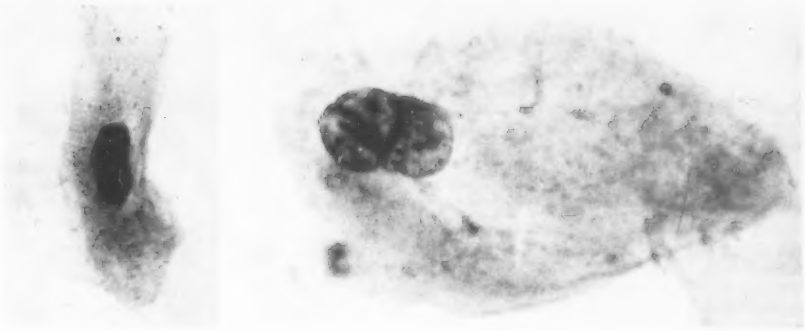


Fig. 1. Superficial dyskaryosis. Note the large hyperchromatic nucleus which is almost completely pyknotic. The normal counterpart of this cell is the superficial cornified cell. (R. M. Graham)

Fig. 2. Superficial dyskaryosis. Two malignant nuclei in a cell whose cytoplasm is normal in amount and configuration. The benign counterpart of this cell is the pre-cornified cell. (R. M. Graham)

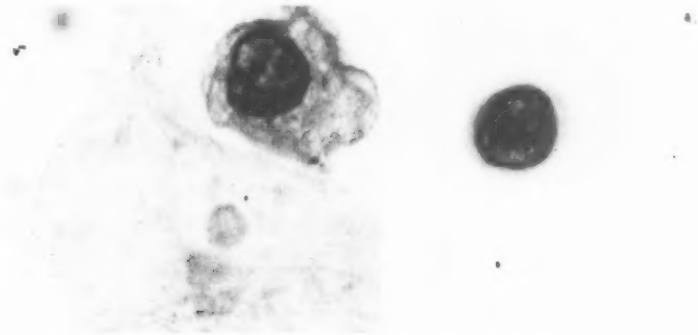


Fig. 3. Basal cell dyskaryosis. A malignant nucleus in a cell whose cytoplasm is still abundant. The normal counterpart of this cell is the outer layer basal. (R. M. Graham)

Fig. 4. Third type differentiated squamous cancer cell. This cell is considered as a true malignant cell though highly differentiated. The evidence for this is that in determining what kind of cancer cell was present in 172 invasive carcinoma of the cervix cases, all stages of disease, this particular cell was present in all but nine cases, 95%. (R. M. Graham)

LEOPOLD G. KOSS (by invitation)*, New York, New York, U.S.A.:

Papanicolaou (1), upon introducing the term "dyskaryosis," describes dyskaryotic cells as follows: "The nuclei show distinct abnormal features such as enlargement, hyperchromasia, anisokaryosis, bi- or multinucleation, etc., but the malignant cells do not exhibit the extreme deviations from the normal types from which they originate, as they do in advanced cases of malignancy." Papanicolaou (loc. cit.) further proceeds to classify dyskaryosis as: superficial cell dyskaryosis, intermediate or navicular cell dyskaryosis, parabasal cell dyskaryosis, and dyskaryosis affecting endocervical cells.

Obviously, any deviation from the above definitions would have to be classified under some different, perhaps not as yet invented term.

I do not know what is meant by "typical malignant nucleus." Observation of neoplastic lesions of the uterine cervix will readily disclose an entire spectrum of nuclear changes which are not always present simultaneously in the same cell. However, as a rule, dyskaryotic cells show larger or multiple and more hyperchromatic nuclei than normal cells of comparable origin.

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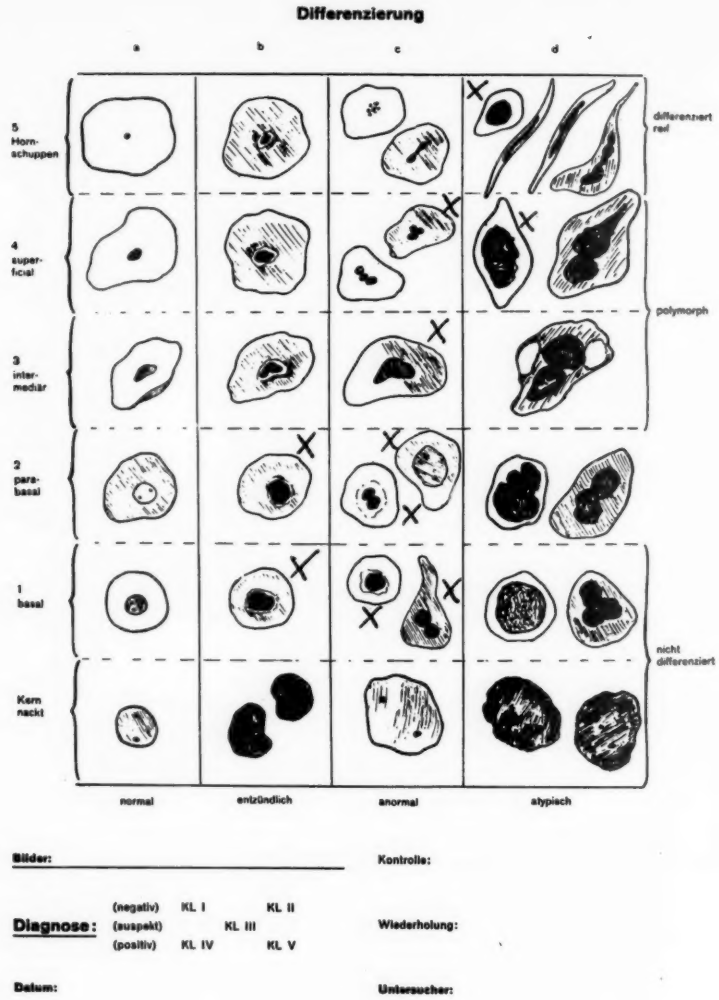

Fig. 1. (Jean Berger)

The problem of "nucleocytoplasmic ratio," a more correct term (2), is a delicate one since this ratio varies substantially in perfectly normal cells according to their degree of differentiation. Thus the range in normal cells may be from 1:6 to 1:2. We have clearly demonstrated (3) that although the majority of dyskaryotic cells fall well within the normal nucleocytoplasmic ratio, in some of the smaller cells the ratio will be reversed in favor of the nucleus and will fall within the 1:1 range. Yet the latter cells may be clearly identified as of parabasal origin and thus fall into the category of dyskaryosis. Consequently, the relationship of the nuclear diameter to the distance from the nuclear border to the cellular border does not apply here either. Moreover, measuring cells, although an accepted method of scientific investigation, would hardly be applicable as a routine procedure in a busy laboratory and would render the practice of cytology exceptionally tedious. I would rather accept accept the visual criteria outlined by Papanicolaou.

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JAMES W. REAGAN, Cleveland, Ohio, U.S.A.:

The contributions pertaining to "dyskaryosis" clearly illustrate the need for standardized terminology in the field of applied cytology and the need for a sound scientific approach if we are to materially advance our knowledge of cells.

The basic scientist who deals with cells might even question the use of the term "dyskaryosis" because it implies that the alteration is confined solely to the nucleus. Actually, the nucleus and the cytosome are not dissociated parts of the cell but are integrated components of the complex unit which constitutes the protoplast. Cytology is by no means restricted to morphology but also encompasses the chemical and functional study of the cell. The absence of significant cytoplasmic changes in the cells which constitute "dyskaryosis" in no way excludes the presence of chemical or functional changes which may be of great significance.

All cells in the human organism originate in the tissues and our only means for determining their lesion of origin is by correlating cellular and tissue changes. Several of the discussants indicate that the cellular changes in question are more commonly encountered in the presence of an altered surface change rather than in the presence of outspoken cancer. This in itself is of considerable importance although one of the authors is critical of the workers, who, like myself, indicate whenever possible the specific histopathological process giving rise to the cellular abnormalities. One of the discussants cites arbitrary standards which are used to distinguish the "dyskaryotic" cells.

The divergent views expressed on "dyskaryosis" serve to indicate the need for basic studies designed to further our knowledge of cellular alterations in various disease states and illustrates the need for a critical evaluation of the entire concept. Our knowledge of cytology can be advanced more rapidly if we devote more time to analytical studies and eliminate insofar as possible the subjective factors involved. Applied cytology is a mature science which should be associated with an acceptable scientific terminology rather than with a nomenclature which implies a total ignorance of the basic science of cytology. This international organization might play an important role in working towards these goals.

JOSE R. del SOL (by invitation)*, Madrid, Spain:

To define some commonly used entities about which there was no general agreement before is always a difficult task. In discussions with many outstanding cytologists from North and South America and from Europe I gained the impression that the term "dyskaryosis" is applied in various laboratories to different cytological features.

In my opinion a dyskaryotic cell is an epithelial cell with normally differentiated cytoplasm containing an abnormal nucleus.

As I read the definition by Dr. Graham, I thought that her cytometric qualification would exclude some of the cells which I would have defined as parabasal dyskaryotic cells. On the other hand, I would like to say that she has here a definite point: those parabasal dyskaryotic cells which exhibit a large dark nucleus with nuclear criteria of malignancy are actually significant for an irreversible malignant lesion, be it an invasive carcinoma or a carcinoma in situ. As the term "dyskaryosis" was actually meant, if I understand it correctly, to classify some atypical cells into a group which is not definitely identified as "malignant," I would think Dr. Graham's cytometric qualification is commendable, although it may be difficult in some cells, to decide on the basis of this cytometric qualification, in which group they belong.

I would like to ask Dr. Graham if she could give us her definition as to what constitutes an intermediate dyskaryotic cell as compared with a superficial dyskaryotic cell. It seems to me difficult to use the pre-keratin content or the keratin content of the cytoplasm as criteria, as dyskaryotic cells often exhibit abnormal keratinization. Is the differentiation then done only according to the size of the cells?

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PETER STOLL, Heidelberg, Germany:

My definition of dyskaryosis is not as broad as that of Dr. Graham.
Definition: A dyskaryotic cell is an epithelial cell showing normal cytoplasmic differentiation but containing a morphologically normal nucleus which is immaturely developed when compared with the other features of the cell.

The following types of dyskaryotic cells are distinguished:

1. Superficial cell dyskaryosis:
   (a) Superficial cells with basal type nuclei.
   (b) Superficial cells with one or more intermediate type nuclei.
2. Intermediate cell dyskaryosis: Intermediate cells with basal type nuclei.
3. Parabasal cell dyskaryosis: Parabasal cell forms with ripe (acidophilic) cytoplasm and basal type nuclei.

The following conditions are necessary to establish the diagnosis of dyskaryosis: (1) the cell as a whole must be well preserved, and well stained, (2) the cytoplasm must be histochemically ripened, (3) the nucleus must be morphologically and histochemically unripe, but normal. For all practical diagnostic purposes dyskaryotic cells are put into the class III of Papanicolaou.

Normal epithelial cells	Not normal epithelial cells	Atypical epithelial cells (carcinoma)
Class I & II by Papanicolaou	Class III by Papanicolaou	Class IV & V by Papanicolaou
	(A) Dyskaryosis (B) Secondary cellular & nuclear changes (such as due to inflammation, or lysis) which do not permit a definite classification to either the right or the left group.	

The finding of dyskaryotic cells according to our interpretation does not necessitate immediate histological examination, but merely repetition of the smears.

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GUILLERMO TERZANO, Buenos Aires, Argentina:

It is hard to define a dyskaryotic cell because this term was used by Papanicolaou to designate an "undefinable" type of cell.

This problem is made harder by the fact that cervical dyskaryotic cells are (for us) much alike, regardless of the nature of the lesion from which they are desquamated. In the case of carcinoma, dyskaryotic cells occur with malignant cells. They are also observed in Trichomonas vaginalis infestation, pregnancy and high estrogenic therapy, etc. Based on the study of dyskaryotic cells only, we find it impossible to arrive at a correct diagnosis.

We contend that a cell should be classified as dyskaryotic when it is not a malignant cell (a Class IV cell) but shows a large, irregular and hyperchromatic nucleus suggestive of malignancy, while the cell looks normal in size and shape. A false positive diagnosis can arise in the presence of dyskaryotic cells, only if they are misinterpreted.

It is our belief that the term dyskaryosis should not be confined to the changes observed in squamous cells alone, since these signs, usually considered as those of dyskaryosis, have been found also in columnar cells.

CLOSING REMARKS:

RUTH M. GRAHAM:

It is obvious from these discussions of the definition of a dyskaryotic cell that there is little agreement of the characteristics of such a cell. It is surprising that so many of the discussions are rather vague and indefinite. Such phrases as "indefinable" or "column c or d" lack the precision usually associated with a scientific discussion. I believe fully that the morphologic characteristics of dyskaryotic cells are susceptible to precise definition.

I agree with Dr. Reagan that the cytologic picture mirrors the changes that are present in the tissue and that orientation of the cytology to histology is necessary if we are to place cellular changes on a firm foundation.

I believe that a precise definition of the cytoplasmic nuclear ratio is essential in defining the dyskaryotic cell. The evidence for considering cells having only a thin cytoplasmic rim as a third type differentiated squamous cancer cell is as follows. In one hundred and seventy-two cases of invasive squamous carcinoma of the cervix differential counts were done on the type of malignant cell present. In one hundred and sixty-three cases, or 95 per cent, the third type differentiated cancer cell was present. Such a correlation as this indicates to me that these are actual malignant cells and should be recognized as such.

On the other hand, in twenty-five cases classified as atypical by histology, twenty-one, or 86 per cent, had the cells which I consider to be dyskaryotic because their malignant nuclei are surrounded by abundant cytoplasm. Therefore, I must conclude that when only dyskaryotic cells are present the pathologist will not regard the lesion as carcinoma in situ, let alone an invasive carcinoma and that these cells must be placed in a separate non-malignant category.

In reply to Dr. del Sol's question as to how I distinguish an intermediate dyskaryotic cell from a superficial dyskaryotic cell, I cannot. I classify dyskaryotic cells in two main groups—superficial

and basal and distinguish between them on the basis of shape, the basal type being round or oval and the superficial having squared-off borders. I am not familiar with any cellular changes which I have been able to identify as endocervical dyskaryosis.

The point raised by Dr. Reagan that the term dyskaryosis might be questioned since it only refers to the nucleus, and the nucleus and the cytoplasm cannot be disassociated, is an interesting one. I agree that it would be in error not to consider the total cell, but since the alterations in the cell appear to be confined to the nucleus as far as we can tell at the present time, I think that the term "Dyskaryotic" fulfills a useful purpose as a purely morphologic term.

In conclusion, I make a plea for more precise evaluation of cellular changes and the submission of objective evidence. What are the relations of these cellular changes to the histologic picture? In how many cases does Dr. Terzano find his "undefinable" cells? Were the histologic pictures equivocal? What correlation with the histologic picture does the unripe benign cell of Dr. Stoll have? I sincerely hope that through the medium of the ACTA CYTOLOGICA we shall be able to place cytologic terminology on a strict scientific basis and thereby have a common language.

* * *

MORPHOLOGY OF THE DYSKARYOTIC CELLS

GUILLERMO TERZANO
Buenos Aires, Argentina

Papanicolaou (4) has designated as dyskaryotic those cells which are abnormal enough to give the impression of malignancy but not sufficiently abnormal to warrant a positive diagnosis. Usually they are classified as Class III (suspicious).

The Cells, as a whole, retain the morphologic pattern of the layer of epithelium from which they arise: superficial type, intermediate type and parabasal type.

The Cytoplasm generally shows no characteristic criteria, except unusual acidophilia, sometimes orangophilia and non-specific vacuolization which may appear as a single large vacuole, several small ones, or one vacuole around the nucleus.

The Nuclei are irregular in shape, lobulated and large, but because of the amount of cytoplasm, the normal nuclear cytoplasmic ratio remains unaltered. There is always an increase in the chromatin content (hyperchromasia), and often anisokaryosis or multinucleation is observed.

Cells exfoliated from the endocervix and from the endometrium are also "dyskaryotic" if they exhibit similar abnormal features.

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3. Lapid, L. S. and Golderberg, M. A.: Am. J. Obst. & Gynec. 61:1324, 1951.
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DISCUSSION:

J. ERNEST AYRE, Miami, Florida, U.S.A.:

In examining the morphology of dyskaryotic cells, the writer is of the belief that greater effort should be made by the gynecologic cytologist to differentiate between the bizarre, hyperactive inflammatory dyskaryotic cell-type and the dyskaryotic cells of premalignant or carcinoma-in-situ type. This differentiation should be based upon a critical evaluation of cell findings rather than to be based upon histological results which may not reveal the "heart" of the lesion and therefore may be inaccurate and misleading. Cytology is a precise science in the uterine cervix. Even so, in diagnostic practice it is mandatory to insist upon histological confirmation preceding therapy. On the other hand, in research studies designed to reveal the true nature of dyskaryotic cells, the experienced cytologist learns to base his judgment upon cell morphology, faciliated by rich concentrations of abnormal cells revealed in the cell smear or scraping.

CLOSING REMARKS:

GUILLERMO TERZANO:

The remarks of Ayre emphasize the importance of being an "experienced cytologist." It requires great experience to distinguish between dyskaryosis caused by inflammatory reactions as opposed to dyskaryosis caused by early carcinoma.

ANALYTICAL STUDY OF THE CELLS IN CERVICAL SQUAMOUS CELL CARCINOMA

JAMES W. REAGAN
Cleveland, Ohio, U.S.A.

This is a study of the morphological characteristics of malignant tumor cells in cytological preparations from 100 women with histologically proven squamous cell carcinoma of the uterine cervix. The cellular characteristics are discussed in relation to the tissue of origin and are compared with the cells originating in the normal cervix and in histologically proven dysplasia and carcinoma in situ. The cellular data was obtained by studying, at random, samples of the abnormal cellular population in cell films from 100 cases. Measurements were obtained by tracing the projected images of 50 isolated cells selected at random and measured by planimetry. The histological preparations were also studied in detail. Statistical analyses were made to evaluate the degree of significance in the cellular characteristics.

The mean cell area of invasive carcinoma was smaller than that recorded for normal cells, dysplasia, or carcinoma in situ. The distribution of this data indicated that a significant number of the cells were essentially of the same size as those in carcinoma in situ. In shape the cells from invasive carcinoma were non-isodiametric in 52 per cent as compared with 39 per cent in carcinoma in situ, 27 per cent in dysplasia and 4 per cent in the normal. Bizarre pleomorphic cells were noted in approximately 33 per cent of the cases of invasive carcinoma and rarely in the other conditions. Sheets of exfoliated cells were seen most frequently in dysplasia, but were significantly more common in invasive cancer as compared with carcinoma in situ. Syncytia were observed twice as often in invasive carcinoma as in carcinoma in situ. They were not observed in the normal or dysplastic state.

The mean nuclear area in invasive cancer was larger than in normal cells but smaller than the mean area of dysplasia and carcinoma in situ. The mean relative nuclear area (nuclear area/cell area) of invasive cancer was calculated to be 34 per cent as compared with 32 per cent in carcinoma in situ, 16 per cent in dysplasia and 2 per cent in the normal. The percentage of non-isodiametric nuclei was greater in the presence of frank cancer than in any of the other conditions. No attempt was made to quantitate hyperchromatism, however, a coarsely granular chromatin pattern was observed in 72 per cent of the nuclei in carcinoma in situ, as compared with 47 per cent in invasive cancer. In dysplasia and the normal only 4 per cent of the cells contained coarsely granular nuclei. Opaque nuclei were observed most commonly in the normal state in the small pyknotic nuclei of senescent cells whereas the opaque nuclei of malignant tumor cells were noted to be larger.

Nucleoli were noted in both carcinoma in situ and invasive cancer, while macronucleoli were seen only in cases of invasive cancer. The analysis of the cellular data from invasive carcinoma indicated the possibility that more than one type of lesion was represented in the group of 100 cases. The cellular preparations were examined with respect to cell size, relative nuclear area and pleomorphism. This analysis resulted in three distinct sub-groups having significantly different cytological characteristics by statistical analysis (a large cell keratinizing carcinoma, a small cell undifferentiated cancer and a large cell non-keratinizing carcinoma). The histological lesions reflected the variations in the cytological preparations.

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* * *

CYTOCHEMISTRY OF DYSKARYOTIC CELLS

H. WERNER BOSCHANN * (by invitation)
Berlin, Germany

The dyskaryotic cells [1,2,3], as discussed below, are squamoid cells with functionally immature and morphologically atypical nuclei, but apparently with normally differentiated cytoplasm. The functional and morphological degrees of maturity of normal squamous epithelial cells may be recognized in the cytoplasm of the dyskaryotic cells.

The techniques [4-10] which I have evaluated are demonstrated in the table at the conclusion of this paper.

The results [10] which were obtained employing these cytochemical examinations are listed below, according to findings in the cytoplasm, nucleus, and nucleolus of the dyskaryotic squamoid cells.

I. CYTOPLASM

A. Parabasal Dyskaryotic Cells

Ribonucleic Acid (RNA) content: abundant (as compared with the de-differentiated cancer cells which are occasionally poor in their RNA content). These dyskaryotic cells, therefore, show genuine basophilia with such substances as Toluidine Blue or Methylene Blue (basophilia may be suppressed by previous treatment of the cells with ribonuclease), and the cytoplasm will be dyed red with Pyronin. All these aforementioned staining methods may be used also as supravital staining procedures.

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Polysaccharides: usually negative. Only in parabasal dyskaryotic cells with a large amount of cytoplasm may one observe a few glycogenic granules (Iodine method, Best Carmine, Periodic acid-Schiff). These granula disappear is the cells are treated with salivary diastase (Ptyalin) or any other kind of diastase.

SH-groups (pre-Keratin) and SS-groups (Keratin): negative.

Lipids: neutral fats as well as acidic lipids may occur in extremely fine granules.

Enzymes: (a) Succinic dehydrogenase: marked activity (at location of mitochondria?). In a controlled reaction using a microscope with a heated stage blue Formazan-purple-granules will appear a few minutes after the onset of the incubation. These granules appear in dyskaryotic cells and in cancer cells earlier than in normal parabasal cells.

(b) Peroxidase: similar to (a), but less reliable.

B. Intermediate Dyskaryotic Cells

The intermediate dyskaryotic cells exhibit less basophilia, less activity of succinic dehydrogenase and peroxidase, whereas the presence of glycogen is increased, as compared with the parabasal dyskaryotic cells.

C. Superficial Dyskaryotic Cells

Basophilia: negative. Pseudobasophilia occurs in non-cornified dyskaryotic cells due to the acidic parts of the polychrome dye of the Papanicolaou staining technique.

The Periodic acid-Schiff reaction is strongly positive. The PAS positive portions of the cells are increasingly resistant to the influence of diastase. There is apparently an increase of mucopolysaccharides, since this diastase-resistant mortance is also resistant to hyaluronidase.

The most mature cell forms exhibit positive reaction for sulphydryl groups (for cysteine in pre-keratin). The positive reaction for disulphide groups, however, is reserved for the most mature forms of cancer cells, the spindle cells.

II. NUCLEOLUS

The nucleolus exhibits a Feulgen positive outline, but is itself Feulgen negative and pyroninophilic. The chromocenters, however, are Feulgen positive and are stained strongly with methyl green.

Cytochemically there is no qualitative difference between nucleoli of dyskaryotic cells and those of normal and cancer cells.

III. NUCLEUS

Marked basophilia (strongly positive Toluidine Blue reaction), strongly positive Feulgen reaction (DNA of all degrees of polymerization), marked methyl green reaction (highly polymerized DNA) with exclusion of the nucleolus is found. The basophilia may be annulled by pre-treatment with desoxyribonuclease. Marked activity of acid and alkaline phosphatase, phosphamidase, and carboanhydrase is present. There is no difference of findings in cancer cells after varying periods of time of incubation. However, there is considerably higher enzymatic activity as compared with normal parabasal cells and parabasal cells from regenerative squamous epithelium.

The latter reactions, which are characteristic of highly active, immature nuclei, persist up to the most superficial layers as contrasted with the progressive cytoplasmic maturation. In the dyskaryotic nuclei of the most superficial cell layers one sometimes finds pyknosis or nuclear masses of the size of 10μ in diameter (the normal pyknotic nucleus is approximately 5μ in diameter) with a perinuclear halo the original size of the nucleus.

CONCLUSIONS

As evidenced by the above cytochemical findings the nuclei of dyskaryotic cells is qualitatively identical with the nuclei of cancer cells. Partial quantitative examinations with varying periods of time of incubation do not permit differentiation of dyskaryotic cells from cancer cells, but permit, definitely, differentiation of both the dyskaryotic and cancer cells from nuclei of cells of the normal or regenerative epithelium. The same findings may be obtained in histological sections.

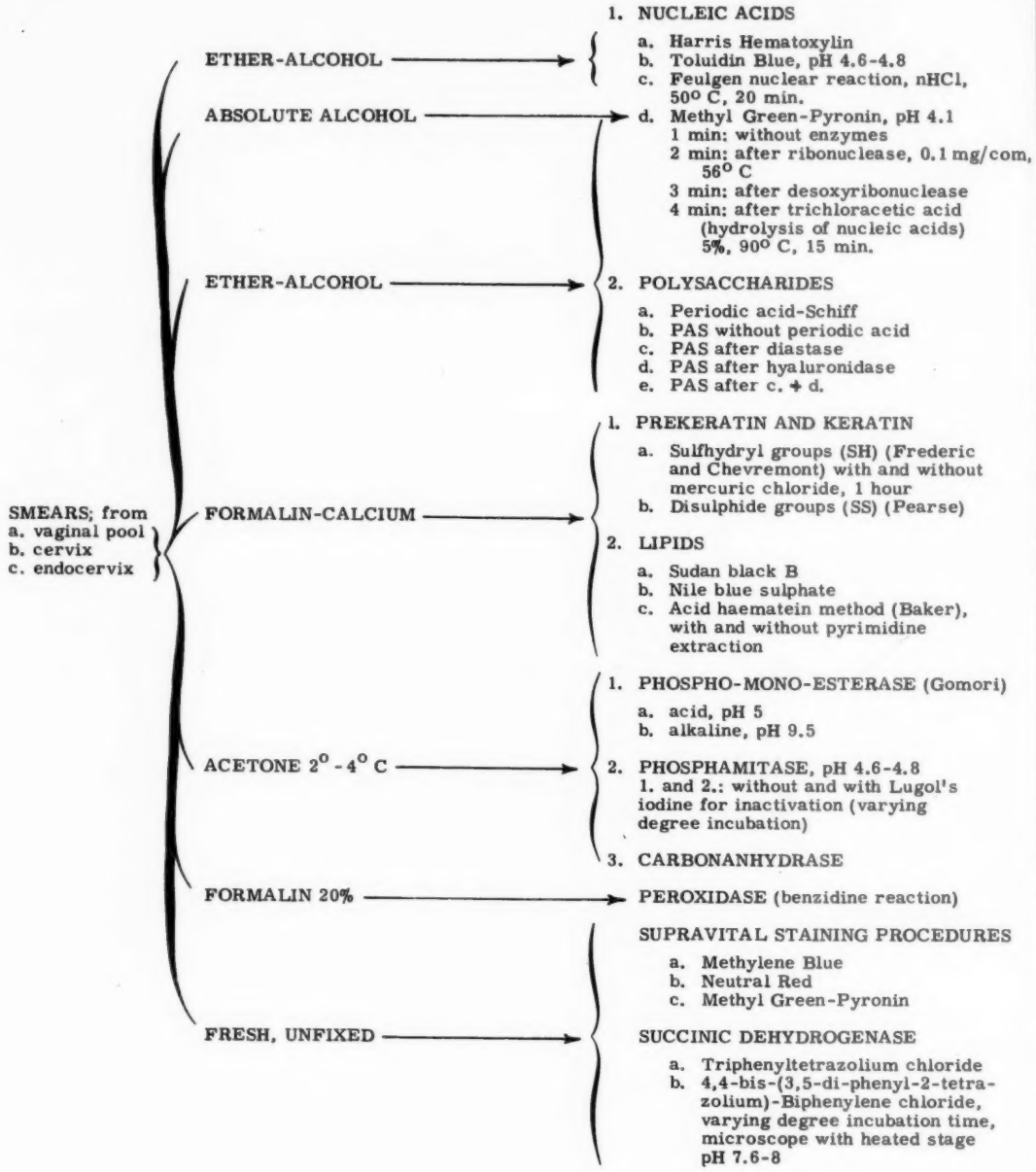
The cytoplasm of the dyskaryotic cell exhibits in cytochemical reactions qualitatively the same process of maturation as the normal cell. The dyskaryotic cell may even be cytolyzed by Döderlein bacilli. The apparently increased activity of succinic dehydrogenase indicates an increase in metabolic intensity over that of the normal cells.

The results of the cytochemical examinations suggest that the dyskaryotic cells may represent the highest degree of re-differentiation which may be achieved by cancer cells.

Thus, one observes a similarity with the highest degree of reversible de-differentiation which may be achieved by the normal cell under inflammatory proliferative stimulation. The distinction of those two kinds of dyskaryotic cells is easier done by the anti-inflammatory test therapy (such as with hydrocortisone) or by estrogen test therapy, than by the qualitative histochemistry. The dyskaryotic cells of the irreversibly changed cell type do not respond to either of the two kinds of test therapy.

Summarizing one may say that cytochemical examinations contribute to our understanding of the appearance of the cells after the staining procedure by Papanicolaou. However, cytochemical studies do not offer any improvement which may be applied to our practical diagnostic problems.

Table of Examined Techniques for Cytochemical Studies



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DISCUSSION:

ROBERT C. MELLORS* (by invitation), New York, New York, U.S.A.:

Boschann has, in this apparently comprensive study, correlated certain morphological and chemical features of dyskaryotic cells in vaginal, cervical, and endocervical smears. The methods used cannot be regarded as absolutely specific for the chemical materials enumerated, and yet they are sufficiently selective (bearing in mind the controls used) to provide a reasonably good approximation to the true state of affairs. Their application to the problem at hand is most commendable, to say the least, in view of the present state of knowledge on this subject. I personally would contest perhaps only one statement that is made by the author in his conclusions, namely, that the results "suggest that the dyskaryotic cells may represent the highest degree of re-differentiation which may be achieved by cancer cells." This appears to me to be only one of perhaps several alternative interpretations, but I have not, of course, had the benefit of hearing the author's total evidence in the matter.

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CLOSING REMARKS:

H. WERNER BOSCHANN:

I am glad that Dr. Mellors commented in regard to the absolute specificity of the presently known qualitative histochemical reactions, since I could not discuss this point in the paper because of the restrictions in the allocated space. Despite the objections to some reactions which are known to everyone familiar with histochemistry it seemed useful to present the spectrum of cytochemical reactions at our present stage of knowledge as a basis for discussions. The alternative interpretation of my findings about the cytochemistry of dyskaryotic cells implied by Mellors makes me anxious to hear his views.

* * *

ULTRAVIOLET MICROSCOPIC OBSERVATIONS ON DYSKARYOTIC CELLS

GEORGE L. WIED
Chicago, Illinois, U.S.A.

Supravital staining of tissues and cells with fluorescent dyes has been practiced for many years (1, 2). Various fluorescent dyes have been used on cytological smears (3, 4). The supravital staining of smears may, however, be accompanied by artefacts due to overstaining or understaining.

In order to avoid such artefacts in the smears, we have administered fluorescent dyes parenterally to the patient during examination. The substances used were Hematoporphyrine (Travenol Laboratories, Inc., Morton Grove, Illinois, U.S.A.) or Acriflavine Neutral (National Aniline Division, New York 6, N. Y., U.S.A.). The patients received either substance by injection while on the gynecological table. The smears were prepared at regular intervals and the cervix visualized by means of an ultraviolet stereocolposcope (Leisegang, Meineke Street 10, Berlin W. 15, Germany). The advantage of this parenteral technique is that one obtains uniformly stained cells. The disadvantage, however, is

that the above substances are not entirely harmless to the patient and, therefore, can not be currently used as a routine procedure. The smears obtained by this procedure are screened under the UV microscope and the actual quantity of absorbed dye measured with an attached photocell.

If one defines a dyskaryotic cell as an epithelial cell with a seemingly normal differentiated cytoplasm and a definitely abnormal nucleus, then one observes under the UV microscope that most of the nuclei of dyskaryotic cells from the deeper squamous cell layers and endocervical dyskaryotic cells, exhibit an absorption of the fluorescent dye which is practically identical with that observed in "cancer" cells. There is usually a characteristic difference in the absorption of the fluorescent dye when normal and "cancer" cells are compared.

This preliminary report is based on the observations from a few cases under examination, but it appears that the nuclei of most dyskaryotic cells (according to our above definition) exhibit an absorption of fluorescent dyes which more closely resembles that of "cancer" cells than of normal cells.

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This study is supported by a grant from the United States Public Health Service (Grant No. CS-9473).

DISCUSSION:

ROBERT C. MELLORS* (by invitation), New York, New York, U.S.A.:

Wied has undertaken the study of cervical and vaginal cells which were stained intravitally by giving patients parenteral injections of fluorescent dyes during the examination. The actual quantity of dye absorbed was determined by measuring the fluorescence of cells with a photocell and microscopic assembly. It was found in preliminary work that relative to normal cells most of the nuclei of dyskaryotic cells exhibited an increased content of fluorescent dye just as did the nuclei of "cancer" cells. These findings appear to be consistent with quantitative data obtained in our study some years ago of the nuclear content of fluorescent dye in cells of Papanicolaou classes I-V in vaginal smears stained (supravitally) with berberine sulfate (Cancer 5: 458-468, 1952).

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JEAN BERGER, Basel, Switzerland:

We agree with Wied that fluorescence microscopy in cytologic cancer diagnosis will promote new aspects. By vitalfluorochroming, Wied could find no significant difference between dyskaryotic and cancer cells under the ultraviolet microscope.

For quite a while we have employed fluorescence-microscopy in the early diagnosis of cervical carcinoma. Our findings agree with those of Wied. The dyskaryotic cells and nuclei show the same fluorescence as cancer cells. Also we find similar pictures when the prepared histologic cuts show marked atypia, "carcinoma in situ" and "invasive carcinoma." In our investigations we used the cathodic Acridinorange fully described by Strugger and collaboraters, as well as the anodic Thiazolyellow. In order to avoid faulty results the following physical-chemical basic properties have to be determined exactly: concentration of the solution, the pH, the time of staining, the influence of the ultraviolet exposure and the medium of solution (for instance glucose or physiologic saline solution). We would like to reiterate that the dissociation of cathodic and anodic fluorochromes is quite different. The isoelectric point of the amphoteric proteins of cells and nuclei is important in staining with fluorochromes. In our experiments we notice that the staining of dyskaryotic cells and cancer cells with Acridinorange is the same. As dyskaryotic cells and cancer cells show the same fluorescence in similar pH-solutions, we may conclude that the iso-electric point is also the same for single substances of these cells. In order to obtain more accurate results we have to stain with fluorescent solutions in ascending pH concentration. The fluorochroming of single cell entities in different pH concentrations should also be done. These basic experiments are very important. Although this is only a preliminary hypothesis, we believe that new possibilities for cancer diagnosis can result from this work.

CLOSING REMARKS:

GEORGE L. WIED:

I would like to thank both Drs. Mellors and Berger for their discussions. I would also like to point out that our study was based on a very few patients, and that our parenteral technique of administering fluorescent dyes is still in its infancy as far as cervical lesions are concerned. I hope that further studies will be carried out and will show the usefulness of this technique for research purposes.

Drs. Mellors and Berger used in their studies supravital staining of exfoliated cellular material which may be easily applied even for routine examinations. The supravital staining procedures, however, produce often artefacts by overstaining or understaining and are sometimes difficult to reproduce. Therefore, by use of the supravital technique one often cannot very accurately determine the actual quantity of fluorescent dye that is absorbed. However, if one injects the dye and stains the cells intravitally one can expect that the dye is more evenly distributed, and that the measurements of the fluorescence of the cells with a photocell and microscopic assembly may be more conclusive.

The supravital staining procedures have the advantage over our intravital technique in that one can perform innumerable experiments with any fluorescent dye and concentration thereof, whereas the intravital methods will be restricted to the few fluorescent dyes which are rather harmless to the patient if parenterally administered.

* * *

DYSKARYOTIC CELLS UNDER THE COLPOMICROSCOPE

T. ANTOINE, K. BRANDL, V. GRUENBERGER, E. KOFLER and H. KREMER (by invitation)*
Vienna, Austria

Dyskaryotic cells may be identified with the colpomicroscope. Our colpomicroscopic definition of dyskaryotic cells embraces those cells which exhibit some of the following criteria: 1) relative enlargement of the nucleus, 2) hyperchromatosis of the nucleus, 3) bi-or multinucleation, 4) perinuclear halo formation, 5) lack of structural details in the nucleus, 6) karyolysis, 7) karyorrhexis, 8) anisokaryosis, 9) pseudomitoses, 10) "bird's eye" cells.

Originally, we did not classify cells with these features as "dyskaryotic," but we identified them as more or less suspicious according to the severity of the atypia. We assume that we deal with a benign process (usually chronic inflammatory changes) if we find the above ten criteria in merely a few cells or if the above criteria are not very marked. We classify findings as suspicious of carcinoma if we find the above atypia to a marked degree, especially the criteria listed under Nos. 1, 2, 3 and 5. These features are even more marked if we can identify definite cancer cells which are characterized by extreme hyperchromatosis, irregularity of the chromatin distribution, pathological mitoses, and extreme anisokaryosis. One of the criteria which we use in colpomicroscopy for the suggestive diagnosis of carcinoma is a considerable increase of the relative number of nuclei per square unit.

In many cases it rests with the individual examiner as to what will subjectively be interpreted as suspicious of carcinoma. This subjective interpretation is a matter of experience in colpomicroscopy, in the same way as is the diagnostic accuracy of classical microscopy a matter of experience. As a rule, it can be stated that the greater the number of dyskaryotic cells found in colpomicroscopy the greater is the probability that one deals with a malignant lesion.

One of the advantages of colpomicroscopy is that one can observe the cells in vivo, within the undisturbed tissue, and that one may repeatedly examine the area for comparative studies.

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* Tassilo Antoine, M.D., is Professor and Chairman of the First University Department of Obstetrics and Gynecology, University of Vienna, Austria; K. Brandl, M.D., is Resident Physician in Obstetrics and Gynecology, First University Department of Obstetrics and Gynecology; Viktor Gruenberger, M.D., is Professorial Lecturer (Dozent) and Co-Chairman (Ober-assistent), First University Department of Obstetrics and Gynecology. E. Kofler, M.D., and H. Kremer, M.D., are Resident Physicians in Obstetrics and Gynecology, First University Department of Obstetrics and Gynecology. Address of Authors: 23 Spitalgasse, Vienna IX, Austria.

DISCUSSION:

GEORGE L. WIED, Chicago, Illinois, U.S.A.:

If I understand correctly, the authors use a nuclear stain (hematoxylin, Pontamine Sky Blue or Evans Blue) to dye the uterine cervix prior to the colpomicroscopic examination since the unstained uterine cervix does not reveal adequate cellular details (1). Staining with hematoxylin, however, will mainly permit the identification of nuclear details.

In our present discussion on dyskaryotic cells we are also concerned with the pre-keratin content and keratin content of the cells since some dyskaryotic cells exhibit abnormal keratinization.

I wonder if the authors would care to elaborate on the possibility of using a staining procedure which might supply some specific information on the cytoplasm. Would the authors also care to comment whether or not the rapid staining procedure of Rakoff (2) might be useful for this purpose?

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CLOSING REMARKS:

TASSILO ANTOINE:

For some time we have used Toluidin Blue (1 g:100 ccm aqua dest.) instead of hematoxylin. We prefer Toluidin Blue because it is more pleasant to use and it stains more rapidly. Naturally all these stains provide only the nuclear details and dye the cytoplasm very slightly. However, we consider the nuclear staining of primary importance. In accordance with the above suggestion by Wied we investigated the staining procedure of Rakoff and found that it provides good demonstration of the cytoplasm in viva under the colpomicroscope. Rakoff's staining technique permits an especially excellent demonstration of the cellular borders. Further investigation is required to evaluate the practical value of this in colpomicroscopy. Concerning the question whether or not one can determine the presence of keratin or pre-keratin in the cells: we could not demonstrate keratin or pre-keratin with either our conventional staining techniques or with the procedure by Rakoff.

* * *

THE UNFIXED DYSKARYOTIC CELL UNDER THE PHASE CONTRAST MICROSCOPE

HANS KLAUS ZINSER
Cologne, Germany

The appearance of the typical and atypical cells as observed on the fresh or supravital specimen under the phase contrast microscope have been extensively discussed. Reports, however, are scarce on phase microscope findings in the dyskaryotic cell. By dyskaryotic we mean a cell whose cytoplasm exhibits the characteristic criteria of the original cell form (such as deriving from the superficial, intermediate, and parabasal layer). The nucleus, however, manifests a definite deviation from the normal appearance and the nuclear content exhibits abnormal structural properties.

Under the phase contrast microscope we distinguish two main types of dyskaryotic cells:

(1) In the first, we find superficial and deeper cells with rather dark nuclei of variable size which do not show any definite details due to its density. The nucleus seems to lie in an optically empty area (halo), giving the impression that the nucleus is suspended in space. Around this optically empty zone we find a ring of dense cytoplasmic granules. The occurrence of pyknosis, therefore, goes hand in hand with some pecularities in the granular distribution in the cytoplasm.

(2) The second form of the dyskaryotic cell displays a light nucleus with a clearly outlined nuclear membrane. The nucleus usually contains a large nucleolus and granular or diffuse chromatin bodies. There is never a perinuclear halo in this type of dyskaryotic cell. The cytoplasm contains several or abundant granules whose density is considerably increased in the perinuclear area.

It may be assumed that one deals here with cells of varying degrees of differentiation. The first type represents the markedly differentiated form, whereas the second type exhibits criteria of poorly differentiated cell forms.

The observations on the unfixed and unstained cells demonstrate cellular details sufficiently well to warrant further studies, especially comparative examinations with the appearance of the fixed and stained cells. (See four figures.)

DISCUSSION:

PETER STOLL, Heidelberg, Germany:

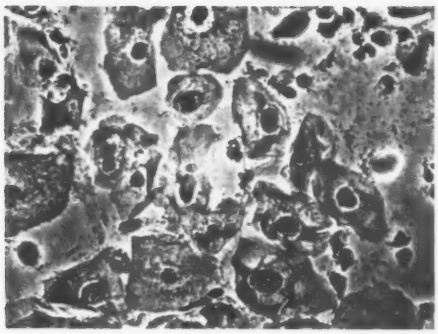
I agree completely with the observations of Zinser and I share his opinion that dyskaryotic (benign) cells can be differentiated under the phase microscope from atypical (malignant) cells. Under phase contrast microscope dyskaryotic cells do not exhibit changes in the form of the cytoplasm, such as amoeboid movement, while malignant cells definitely show such movement. This observation described by Zinser, may be utilized for the differentiation of the second cellular type, from that of malignant cells. In the first cellular type, described by Zinser, the occurrence of the perinuclear halo indicates a tendency towards maturation with shrinking of the nucleus. This first cellular type is, in my opinion, already established in the process of normal maturation. One might conclude therefore that:

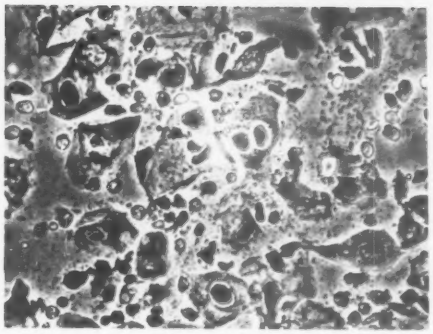
(1) Zinser's Type 1 is a dyskaryotic cell type with tendency towards maturity, and
(2) Zinser's Type 2 is the characteristic dyskaryotic cell type.

CLOSING REMARKS:

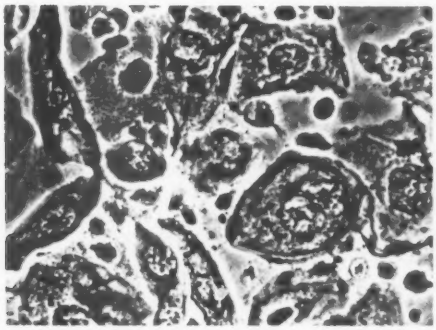
HANS KLAUS ZINSER:

I agree with the findings of Stoll. The first form may be the re-differentiated type. However, I would not venture to classify them as benign. It seems to me that it is rather an attempt towards normal differentiation which is not successful. One should adhere to the concept that dyskaryosis is a process of anaplasia.





Figs. 1 and 2. Dyskaryotic cells in smears of a patient with carcinoma in situ of the uterine cervix. (H. K. Zinser)



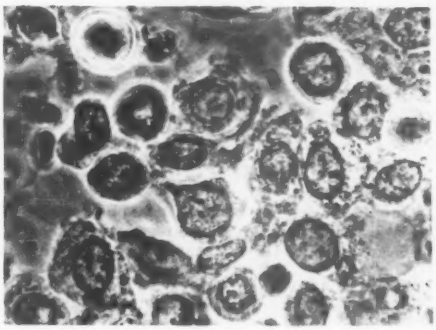


Fig. 3. Dyskaryotic cells in smears of a patient with carcinoma in situ of the uterine cervix. (H. K. Zinser)

Fig. 4. Atypical cells in smears of a patient with invasive cervical carcinoma. All four figures are prepared under the phasemicroscope on unstained and unfixed cytological material. (H. K. Zinser)

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OCCURRENCE OF DYSKARYOTIC CELLS IN TRICHOMONAS INFESTATION

JEAN de BRUX and HENRIETTE WENNER-MANGEN
Paris, France

Severe diagnostic errors in the cytological diagnosis of cancer have been attributed to the presence of Trichomonas vaginalis. Because of this occurrence of cytological atypia in the presence of trichomonads, the cytologist hesitates to give a definite diagnosis.

The factors giving rise to this uncertainty are:

1. The multiplicity of morphological modifications associated with Trichomoniasis.
2. The fact that little is known about the inter-relationship between epithelial abnormalities and the presence of the parasites.

There does not exist a really constant relationship, cytologically or clinically, between the gravity of cellular atypia and the duration of Trichomonas infestation. The frequency of Trichomonas infestation (25% according to Pundel) when compared with the relative infrequency of dyskaryosis would leave one to suppose that its role is not fully understood. It may very well be that the cellular atypia is due to organic factors as well as intrinsic causes.

Cellular Changes:

The presence of Trichomonas vaginalis does not always cause epithelial atypia. As a matter of fact, one may find entirely normal cellular features and normal Doderlein bacilli without inflammatory

cells. This latter finding is observed in about 8% of the cases.

The most frequent findings, among these cellular changes, are hyperactive and degenerative processes, and one particular characteristic of this parasitosis is an abnormal maturation.

The appearance of the smear is characterized by its polymorphism: one finds squamous cells from all layers, as well as glandular cells and metaplastic cells, which are evidence of proliferative and destructive processes in the cervical epithelium. The cytological changes vary widely; some of the most common are:

(1) hyperchromasia, (2) bi-or multinucleation, (3) prominent or sometimes multiple nucleoli, (4) gross distribution of chromatin and so on. Other cellular changes which are more characteristic are: (1) acidophilia or orangeophilia of the cytoplasm of the intermediate and parabasal cells, (2) nuclear alterations: marked density and uniformity of the chromatin structure (similar to karyopyknosis) with the difference that these altered nuclei are often increased in size, round or oval in shape, and rarely irregularly outlined. Less common changes are the frequent occurrence of karyopyknosis in intermediate cells with acidophilic cytoplasm which gives rise to incorrect hormonal evaluations of the smears, (3) a clear perinuclear halo, which, although not absolutely pathognomonic, appears with such frequency that its detection often precedes that of the parasite itself. Its significance is difficult to define. A consensus of opinion suggests that it results from the contraction of the nucleus. The coloration with P.A.S. staining is slightly positive, but disappears following incubation with amylase which suggests that it could contain glycogen.

Histological Correlation:

Biopsies done on the cervico-vaginal mucosa show similar abnormalities. The parasites affect the cellular maturation. The deeper layers of the epithelium often show abnormal activity and increased mitoses. Such cellular anomalies as precocious cornification of the cytoplasm, perinuclear halos, and large, but dense, nuclei, previously described for cytological smears, make their appearance.

These abnormalities may be very marked, and this explains why the presence of parasitosis often gives rise to misinterpretations in differentiating these cellular changes from those occurring as a result of malignant lesions, menopausal atrophies or metaplastic processes. Little is known of the action of Trichomonads on cellular morphology. It appears to act on the sulphydryl groups and disulphide groups bound to proteins, and to affect the activity of dehydrogenase which is concerned with cellular respiration and, in particular, the oxydation of the lipids, carbohydrates and proteins.

Summarizing one may say that there exists a multiplicity of morphological cellular changes, histologically confirmed, which may be attributed to Trichomonas infestation, and that these atypia cannot only be difficult, but actually defy definite classification. However, the great interest in these observations lies more in the academic aspects of the study of the metabolic mechanism of Trichomonads on the cervico-vaginal epithelium.

CLARICE DO AMARAL FERREIRA
Rio de Janeiro, Brazil

The published reports of Trichomoniasis in vaginal cytology describe inflammatory reactive changes in the epithelial cells.

Bechhold and Reicher (3) have pointed out that these changes appear first in the superficial layers and then extend towards the deeper cell layers, distinct from the changes of carcinoma in situ which progress from the deeper to the superficial layers. C. H. Davis (4), who has extensive experience in this subject, reports an incidence of 7.1% Trichomoniasis in 5,115 private patients examined. In this series 16.3% belong to group I, 15.9% belong to group II and 11.8% to group III. The above groups are as classified by J. E. Ayre. With regard to malignancy 0.66% of the total number of the cases with Trichomoniasis proved to be malignant. In a second series of 2,584 cases, which he reported from the Miami Clinic, the incidence of Trichomoniasis is given as 24.9%, with 33% belonging to group I, 27.4% belonging to group I, 27.4% belonging to group II and 12.3% belonging to group III. The incidence of malignancy of this series of cases was reported as 2.21%.

The incidence of Trichomoniasis in our recent series of more than 20,000 cytological examinations performed at the Gynecological Hospital of the University is not presently available. However, in a statistical study published by us in 1955 (1) we reported a 7.5% incidence of Trichomoniasis in 744 private patients of Professor Arnalda Moraes. Of these cases 82.7% were classified as group II and 11.6% were classified as group III (dyskaryotic) by Papanicolaou's method of classification. In a further article (2) presented in 1956 reporting on patients from the Cancer Prevention Clinic and some of the other groups of patients, we compared the frequency of group III findings in Trichomonas vaginitis and chronic cervicitis. Dyskaryotic cells were reported in 11.6% of 69 cases of Trichomoniasis, in 7.4% of 27 cases of mycoses, in 8.4% of 438 cases of chronic cervicitis and in 3.3% of 6456 cases examined at the Cancer Prevention Clinic.

From the above figures it is obvious that there is a high incidence of dyskaryotic cells in cases with Trichomoniasis, but I do not remember finding cellular changes atypical enough to be classified as group IV by Papanicolaou's method of classification.

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JULIETA C. de LAGUNA
Mexico, D. F., Mexico

In our experience, 40% of the patients with Trichomonas show no other cytological changes. In most cases, minimal easily reversible alterations, of superficial and intermediate cells are observed (Class II Papanicolaou classification); eosinophilia, pyknosis, perinuclear halo, and moderate nuclear enlargement in basal cells, (cervicitis, thickening of the epithelium) (Ayre 1, Bechhold and Reicher 2, Laguna et al 3). These alterations produce a picture that could be called "minimal dyskaryosis," sometimes accompanied by endocervical epithelial changes, similar to glandular hyperplasia.

Fifteen per cent of patients show more severe changes, Class III of Papanicolaou: this class includes true dyskaryosis (anaplasia of the epithelium, with cellular atypia) and other alterations due to potentially regressive lesions. We make a separate group of those with dubious malignancy, (doubtful Class III of Papanicolaou) constituting 3% of all cases (Laguna et al 4). Most of the cases in this group have severe glandular hyperplasias, not squamous dyskaryosis. Furthermore, a picture of squamous metaplasia can be identified, but the peculiar intermediate and basal cells, constituting dyskaryosis, should be considered as an independent entity.

In a few cases, alterations in the deep basal layer allow the diagnosis of basal cell hyperactivity. This is the less common but the more persistant of changes; this should also constitute another independent group.

Only 2% of the patients show true superficial dyskaryosis confined to superficial and intermediate layers. Half of them are due to the co-existence of Trichomonas vaginalis with carcinoma in situ. Most of the remaining lesions are regressive.

After consideration of the follow-up examinations, we doubtfully implicate Trichomonas vaginalis in persistent or progressive metaplasias and superficial dyskaryosis, and consider them as not having any bearing on the etiology of the basal cell hyperactivity and carcinoma in situ groups.

Our cytological data has been checked with histological studies.

In our series of 727 patients with Trichomonas vaginalis, the following three grades were established, and are compared with similar groups of women free of Trichomonas infestation.

	Trichomonas vaginalis	No Trichomonas vaginalis
Negative I	38.4	54.5
Negative II	46.5	33.0
Negative III	15.1	12.5

Footnote: We divide Class II of Papanicolaou into two groups: Negative II and Negative III. Negative II is characterized by minimal lesions due, in most cases, to inflammatory processes. Negative III represents more severe alterations of the precancerous type.

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DISCUSSION:

LEOPOLD G. KOSS* (by invitation), New York, New York, U.S.A.:

Bechhold and Reicher's paper on cytologic changes associated with Trichomonas vaginalis infestation brought about a series of investigations, one of which was conducted in our laboratory, concerning the possible relationship of Trichomonas vaginalis and cervical cancer.

The problem can be posed in two ways:

1. Are neoplastic lesions of cervix epithelium ever associated with Trichomonas vaginalis infestation?
2. Can Trichomonas vaginalis produce lesions simulating cervix cencer?

The answer to the first question is yes, since trichomonas is an extremely common inhabitant of the lower genital tract. The answer to the second question is negative in our experience and there is no evidence in the literature that Trichomonas vaginalis was ever seriously considered as a genetic factor in cervical cancer. I was glad to see that de Laguna appears to share this point of view.

There remains the matter of interpretation of the cellular findings in Trichomonas vaginalis infestation. I do not believe that umcomplicated Trichomonas infestation is ever associated with true dyskaryosis. A certain amount of hyperchromasia and, rarely, nuclear enlargement, may be associated with Trichomonas vaginalis, but these findings in our experience correspond to a rather peculiar form of squamous metaplasia of the endocervical epithelium encountered with considerable frequency in association with the parasite. The metaplastic epithelium, very vascular, is composed primarily of somewhat elongated cells forming a pattern of loose "juicy," squamous epithelium. The slightly atypical cells found in smears can be readily compared with the epithelial cells forming the metaplastic epithelium. This lesion is by no means typical of Trichomonas vaginalis infestation and may be also seen in other forms of subacute and chronic cervicitis. There is no reason for either cytologic or pathologic confusion of these lesions with neoplastic lesions of the cervix.

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CLAUDE GOMPEL, Brussels, Belgium:

Our recent experience with Trichomonas vaginalis infestation is based on a study of 1,850 patients seen in 1956. Trichomonas vaginalis was found in 6.1% of our cases. This rather low incidence, if compared with other reports, can be explained by the fact that these patients come from the Cancer Detection Clinic of the Institute and not from a Gynecological Clinic.

We briefly summarize the morphological data which are important to us.

Eosinophilia and perinuclear halos are the most important and typical cellular alterations. They are present in almost every case. The hormonal status or the degree of the infestation does not influence the presence of these alterations. Patients developing Trichomonas infestation after bilateral oophorectomy and bilateral adrenalectomy show a high percentage of cornified cells. Mild clinical infections with a small number or parasites show definite perinuclear halos and eosinophilia.

Nuclear abnormalities are mostly manifested in intermediate and basal cells. Altered superficial nuclei are the result of earlier structural modifications.

The number of polymorphnuclears and, to a lesser degree, the number of histiocytes and lymphocytes in the smear will give a better evaluation of the infestation than the number of parasites. This would support the idea that the atypia are not mainly due to a direct action exerted by the Trichomonas but to secondary inflammatory changes of the epithelium.

We have never encountered atypia due to Trichomonas infestation which could be confused with typical cancer cells of the cervix (Classes IV & V of Papanicolaou).

Cellular changes associated with Trichomonas infestation cannot, in our opinion, be confused with cancer but the idea that chronic infection due to Trichomonas vaginalis could be a favorable factor in the genesis of cervical cancer is more debatable.

The classification of our Class II cases seen in 1956 by Papanicolaou's method shows that 5.6% belong to Class III and 94.4% to Classes I and II.

J. ERNEST AYRE, Miami, Florida, U.S.A.:

The relationship between presence of Trichomonas infestation and dyskaryosis is a subject of wide interest which provides a significant avenue of research into the effects of stress or irritation upon epithelial cells of the cervix. After exploring this provocative investigative problem for several years, the writer is of the opinion that much remains to be learned concerning affects and possible carcinogenic influence, exerted upon cervical cells by this form of parasitic infestation. It seems apparent that there is a wide divergence of opinions and some confusion as to what type of cell is benign and reversible as contrasted to proven malignancy when cytology smears and scrapings are studied in patients suffering from trichomoniasis. The latter have the advantage of proof of origin so helpful in differentiating exfoliating vaginal cells from cells of more deep-seated cervical lesions.

While some patients are unaware of the presence of their Trichomonas infestation and in such patients cells may show little or no hyperactivity, the vast majority of patients exhibit marked pruritis, vaginitis and cervicitis, and well marked morphological cell changes. In some cases, benign inflammatory cells show such bizarre changes as to be confused with neoplastic cells. Further confusion results when actual carcinoma-in-situ is involved in a super-added trichomonas infestation. Some cytologists err in the conclusion that the dyskaryotic (actual in-situ cancer) cells represent pseudo-cancerous but benign reversible cells. This condition may seem to be borne out since some lesions may seem to disappear following biopsy.

In our experience, the trichomonad will accentuate what ever cell pathology is present in the cervix. Most cases showing non-inflammatory dyskaryotic cells, cells of the "precancer cell complex" type, or those characteristic of carcinoma-in-situ, represent actual early malignant cells and are not pseudocancerous inflammatory cells produced by the trichomonad.

The importance of cytology as a tool of research is well demonstrated in exploring this fundamentally important interrelationship. The discovery of doubtful cells in trichomiasis provides a challenge to investigators to evaluate their nature based upon their behaviour. Medical treatment permits eradication of the parasite without removal or disturbance of diseased cervical tissues. At the same time, thru serial cytology studies, morphological cell behaviour changes resulting from its eradication may be closely observed. This enables classification of dyskaryotic cells which are benign and reversible in contrast to those of early malignancy as found in pre-invasive cancer.

JOHN F. FIORINO* (by invitation), Everett, Washington, U.S.A.:

The cytological findings in trichomoniasis have been lucidly described. It is generally agreed that trichomoniasis can alter cytological changes thus causing errors or confusion in the detection of early uterine carcinoma. How these changes are produced still needs clarification. The biochemical approach to this perplexing subject of trichomoniasis has interested me for many years. It is routine procedure to evaluate the vaginal secretions by a Beckman potentiameter, using specially devised glass electrodes. The local epithelial changes produced by a bacterial flora of many different bacterial forms, excluding the Döderlein bacilli, thus producing the optimum environmental habitat for the trichomonad which ranges from pH 5 to pH 6.2. With the restoration of the vaginal pH to its normal range of pH 4 to pH 4.5, the vaginal epithelium becomes reversible and the component cells again appear to be normal. The pitfalls produced by the trichomonad must be avoided and before a definitive diagnosis of an early malignancy is given, the trichomonad should be eliminated.

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CLOSING REMARKS:

JEAN DE BRUX:

Cellular anomalies associated with Trichomonas vaginalis are not severe enough to be interpreted as malignant by an experienced cytologist, but when the inflammatory reaction is pronounced and occurs simultaneously with a cervical lesion, some doubt may remain. A therapeutic test should be performed before giving a final answer.

In the Department of Gynecology of the Broca Hospital in Paris we very rarely give a Class III reading (0.3% over the period 1954-1956 in a total of 5000 patients).

I share the opinion of Laguna and Koss with respect to the frequency of the role of metaplastic changes in ectropia or ectopies in women with Trichomonas vaginitis. I believe, however, that the parasite has a pronounced effect on the morphology of both squamous and columnar epithelium.

On the squamous epithelium Trichomonas produces: (a) a different maturation of the nucleus from that of the cytoplasm; (b) delayed onset of pyknosis and distinct hyperchromatosis, and (c) precocious maturation of the cytoplasm with formation of a perinuclear halo.

These characteristics are particularly marked at the level of superficial and intermediate cell layers. Activity of the deep cell layers, as I mentioned in my original paper, is never marked and can never be considered a dysplasia of the type described by Reagan. The difficulty in interpretation is accentuated when dealing with metaplastic cells from such lesions as ectropia or ectopies, which usually occur at the squamous-columnar junction. The changes, however, brought about in these cells by a Trichomonas infestation differ quite clearly from those cells seen in non-parasitic inflammatory reactions.

In any case we do not accept the term "alterations of the precancerous type" used by Laguna, nor the "pre-cancer cell complex" of Ayre, although we would agree with the latter that Trichomonads will accentuate what ever cell pathology is present in the cervix.

CLARICE DO A. FERREIRA:

No comment.

JULIETA DE LAGUNA:

I thank Dr. Koss for referring to my paper. I am glad we agree there is no carcinogenic effect attributable to the trichomonads. There remains, however, a small percentage of patients (1% in our series of Trichomoniasis) where the cellular alterations produced by the trichomonads are so bizarre as to be confused with neoplastic cells.

Our recent experience after prolonged follow-up of these cases cases has shown that the reversibility of such changes depends more on the systemic anti-inflammatory treatment than on the specific anti-Trichomonas measures taken, and there is not always a correlation between the degree of inflammatory cellular changes and the presence or absence of parasites. There is no objection to medical treatment of these infections before stating a final opinion.

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DYSPLASIA OF THE UTERINE CERVIX

JAMES W. REAGAN
Cleveland, Ohio, U.S.A.

The term dysplasia is used to designate a group of heteroplastic lesions characterized by an increase in the number of immature cells and by abnormal differentiation. This study is based on the cytological details and measurements from 100 cells selected at random from cellular preparations. The specimens were from 100 women with histologically proven dysplasia. Histological studies were used in an attempt to correlate the cellular and tissue changes. The results of this study were compared with similar data from carcinoma in situ and invasive cancer.

The dysplastic lesion occurs in close proximity to the external cervical os, although it may involve the mucosa of the endocervical canal or portio vaginalis. The histological change is described in detail.

A study of the cellular specimens indicates that these changes may be correlated with the histological alteration. The number of abnormal cells observed in the presence of dysplasia is significantly less than from carcinoma in situ. This has been interpreted as evidence of greater maturation in dysplasia as compared with carcinoma in situ. The mean cell area in dysplasia was less than that of the normal squamous cells, but significantly larger than that of carcinoma in situ or invasive cancer. In dysplasia 73 per cent of the cells were isodiametric in shape as compared with 60 per cent in carcinoma in situ and 48 per cent in frank cancer. Although sheets of cells were common, syncytia were significantly more frequent in carcinoma in situ and invasive cancer as compared with dysplasia. Anucleate squames from hyperkeratosis seen in 44 per cent of the cases and cells interpreted as evidence of superficial parakeratosis were present in 84 per cent.

In general, the nuclear mass gives evidence as to the maturity of the cell. The mean nuclear diameter in the dysplastic cell was noted to be twice as large as that of the mature squamous cell and slightly larger than that of carcinoma in situ and invasive cancer. The relative nuclear area (nuclear area/cell area) or dysplasia was larger than that observed in normal cells, but significantly less than that recorded for carcinoma in situ. A finely granular chromatin pattern was characteristic of the nuclei in both normal and dysplastic cells whereas a coarsely granular or opaque pattern was more common in carcinoma in situ and invasive cancer.

The prevalence of proven dysplasia was 0.77 per cent which is consistent with the general prevalence of 0.6 per cent as reported elsewhere. Both dysplasia and carcinoma in situ are twice as frequent in the Negro as compared with the white patient. The mean age of detection in this study was 34 years as compared with 41.5 years for carcinoma in situ.

Although dysplasia is a common finding in the presence of both carcinoma in situ and invasive cancer, the actual relationship has not been established. It has been observed that 60 per cent of the lesions apparent on initial biopsy are not demonstrable in subsequent studies. This may be due to removal of the lesion during the biopsy procedure or to actual regression, which is not uncommon in pregnancy. Multiple punch biopsies or sharp knife conization do not invariably remove the dysplastic lesion. Carcinoma in situ has been observed in about 10 per cent of cases which were previously noted to have histologically proven dysplasia. However, it cannot be definitely established that the in situ lesion was not present earlier. Only one patient with dysplasia has developed invasive cancer.

Cellular and histopathologic changes analogous to those of dysplasia in the human have been frequently observed as an early stage in carcinogenesis in the mouse cervix. This alteration was present in over 90 per cent of all animals who ultimately developed invasive carcinoma.

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OCCURRENCE OF DYSKARYOTIC CELLS IN DYSPLASIA OF THE UTERINE CERVIX

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

I am not certain of what exactly is meant by dysplasia. I trust Dr. Reagan has defined it. We use the term "atypical epithelium" for atypicalities of the cervical squamous epithelium which are abnormal but do not fulfill all the criteria for carcinoma in situ. We have recently done differential counts on the vaginal smears of 25 cases classified as atypical epithelium histologically. Twenty-one or 86% had dyskaryotic cells. If the squamous epithelium is atypical a large percentage of the cases will have dyskaryotic cells. Since the dyskaryotic cells have adequate cytoplasm, it is apparent that there will be no picture of crowding of abnormal nuclei in the histologic preparation, and the pathologist will not regard the lesion as an in-situ carcinoma. Smears which contain only dyskaryotic cells should therefore be classified in Group III, suspicious or doubtful—never as positive.

DISCUSSION:

JEAN BERGER, Basel, Switzerland:

In the histopathological diagnosis of stratified squamous epithelium, we use Hinselmann's classification and refer to "slight" and "markedly atypical" squamous epithelium, but not the term "Dysplasia." Our term "markedly atypical epithelium" corresponds to Glatthaar's and Graham's term "atypical epithelium" and refers to the condition wherein the successive epithelial layers lose their identity and the nuclei display polymorphic forms. These modifications alone, however, are not sufficient to satisfy all the criteria for a real carcinoma. In "marked atypia" we find dyskaryotic cells of the deep, middle or superficial layers varying according to age and hormonal influences. In a total of 160 cases of increased atypical epithelium, we found dyskaryotic cells in 80% of the cases. The direct cervical smears showed a higher frequency of dyskaryotic cells than the vaginal smears (43%). Since the atypical changes in the cells and nuclei were not pronounced, we classified 85% of the cases in Class III and the remainder in Class IV because of co-existent inflammatory components which result in further atypical manifestations. The cytodiagnosis has as its primary objective the constant control of such problems as the above. Atypical epithelial changes are more satisfactorily identified by cytodiagnosis than by colposcopy alone.

JEAN DE BRUX, Paris, France:

If one defines "orthoplasia" as a state in which the epithelium shows strictly normal architecture and cellular morphology, then one can define "anaplasia" as a state in which the epithelium has lost its ordinary structure and its normal cellular regularity. Further, one might define "dysplasia" as a state in which the epithelium is characterized by: (a) abnormalities of architecture, and (b) cellular anomalies, the appearance of which points to a definite disturbance, but without criteria significant for cancer.

1. Dysplasia may show cellular regularity with a slightly altered architecture, as the cellular elements—although situated abnormally show no great individual abnormality. (a) Dysplasias in which cellular maturation is normal or excessive (leukoplasia) with insufficient pyknosis, (b) dysplasias in

which the cellular maturation is arrested at the level of the intermediate cell layers (tardy or insufficient cellular maturation), (c) dysplasias with cellular hyperactivity of the deeper cell types and the beginning of maturation in the superficial layers (benign dysplasia, but active).

In smears the cellular elements may appear as snake-fiber cells, tadpole cells, parabasal cells with more or less cornification, intermediate cells with large nuclei, but without a definite nuclear-cytoplasmic disturbance. These cytological features would be always classified as Class II of Papanicolaou.

2. Dysplasia with disturbed, irregular architecture and with nuclear anomalies (markedly atypical epithelium by Hinselmann).

The question which arises in these dysplasias is: Does the lesion regress, progress or will it persist? Histologically, we would estimate that the more marked the hyperactivity of the basal zone, and the higher these parabasal elements grow in the epithelium without any tendency to maturation, the more suspicious is this type of dysplasia for malignancy.

In cytological smears we place reliance on the following criteria: parabasal cells with large nuclei and without a tendency to cornification, mature cells (snake or fiber cells, tadpole cells, or "pre-cancer cell complex" cells).

It is here that the histologist as well as the cytologist will find it difficult to answer the question: Is this lesion a carcinoma or not and what is the suggested therapy of choice?

LEOPOLD G. KOSS* (by invitation), New York, New York, U.S.A.:

I suspect that dysplasia is closely related to in situ carcinoma and that only the degree of differentiation separates the two lesions. This difference is surely not fundamental. Dyskaryosis is present in both dysplasia and in situ carcinoma. However, the concept of dysplasia provides us with a very useful tool to study the natural history of early neoplastic lesions of the cervical epithelium, and the results of a long range follow-up, presently conducted by Reagan, will be eagerly awaited by all interested in cervix cancer.

The statement by Graham that the pathologist will not regard lesions shedding only dyskaryotic cells as an in situ carcinoma pertains apparently to only one school of thinking and is certainly not representative of other equally valid trends. The same, of course, applies to the degree of suspicion that can be expressed in the presence of dyskaryotic cells. But perhaps these differences of opinion are due to differences in the concept of dyskaryosis which does not appear to be the same.

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EDMUND SCHUELLER, Vienna, Austria:

The term dysplasia seems to me to be rather broad and not distinct enough. Among the atypias of squamous epithelium not severe enough to be classified carcinomas in situ, basal cell hyperplasia seems to be the most important. With too vigorous a scraping with a spatula one may sometimes find in cases of basal cell hyperactivity abnormal cells which are suspicious of carcinoma in addition to dyskaryotic cells. In some of the cases the smears have had to be classified as Class IV (Papanicolaou) and the lesion biopsied to decide whether or not there is evidence of malignancy. If one finds only dyskaryotic cells in smears which have been properly preformed the smear should be classified only as Class III (Papanicolaou). The patient should be kept under cytological surveillance until the smears exhibit either less or more marked atypical features, thus directing the way of therapy. Smears have been repeated in these cases approximately every third month. In senile women one finds sometimes dyskaryotic cells to a greater or lesser extent but these findings do not warrant any further diagnostic procedures in this age group.

GUILLERMO TERZANO, Buenos Aires, Argentina:

In our series there are dyskaryotic cells in the smears of some cases in which the pathological report read: "atypical non-malignant epithelial lesions," especially in pregnant women, after diathermo-coagulation of the cervix, and in cervicitis.

We were prevented from making a false positive report only because dyskaryosis was not observed in cells of the parabasal type, but was confined to cells of the superficial and intermediate type.

CLOSING REMARKS:

JAMES W. REAGAN:

On reviewing the contributions pertaining to "dyskaryosis," it is apparent that there is some agreement as to the general significance of the cellular changes although certain differences in opinion are expressed, as might be anticipated in any consideration of this still controversial issue.

Cells having the features of those described by Papanicolaou under the heading of "dyskaryosis" are observed in the presence of lesions classified as dysplasia. Graham indicates that 86 per cent of the cases classified as having atypical epithelium show cells of this type and Berger observed such cells in 80 per cent of the cases having increased atypical epithelium. Terzano comments on the occurrence of such cells in the presence of atypical non-malignant epithelium while De Brux considers the histocytological correlation in detail. Several of the discussants also indicate that cells having the features of those designated as "dyskaryosis" may be observed in the presence of more serious changes in the uterine cervix.

A standard nomenclature pertaining to the alterations in the desquamated cells and to the parent lesion of origin would be of great value if we are to increase our knowledge of Applied Cytology and are to further our knowledge of cellular changes in various disease processes. The basic scientist who deals with cells might even question the applicability of the term "dyskaryosis" because it implies that the change is restricted to the nucleus. Actually the nucleus and the cytosome are not dissociated components but are integrated parts of the cell. The absence of significant morphologic change in the cytoplasm in no way excludes the presence of a chemical or functional change which may be of great significance in the cells under consideration.

Schueller points out that the term dysplasia is rather broad and apparently favors the heading of "atypia" which is used in many institutions to describe a similar group of related changes in the mucosa. Koss mentions the possibility that the cytological differences between dysplasia and in situ cancer may be due to differences in the cellular differentiation of one and the same basic process. This certainly must be considered in view of our present knowledge.

Ayre, among other discussants, emphasizes the need for furthering our knowledge of "dyskaryotic" cells and with this statement we are in complete agreement. He further indicates the need for distinguishing between lesions which are presumably unrelated to cancer and those which may antedate the development of cancer. The proposal that this distinction should be based primarily on cellular evidence rather than on histological evidence would not be acceptable to most scientists. All cells have their origin in the tissues and any change in the desquamated cells must be explained on the basis of changes in their parent tissues.

The opinions expressed by the discussants indicate that there is basic agreement about the cellular concept of "dyskaryosis," although there is some difference of opinion as to the significance of these cellular changes as evidenced by the differences in reporting these abnormalities. The need of a standardized terminology is clearly evident in the various contributions and this is applicable both to the cell and to its lesion of origin. This body might play an important role in standardizing cellular nomenclature which is used in Applied Cytology.

RUTH M. GRAHAM:

It is of interest that there is more agreement in discussion of the type of cell found in dysplasia than in the definition of the cell itself. Reagan states: "The relative nuclear area (number area/cell area) of dysplasia was larger than that observed for normal cells, but significantly less than that recorded for carcinoma in situ." Another discussant: "With too vigorous a scraping with a spatula one may sometimes find in cases of basal cells hyperactivity abnormal cells which are suspicious in addition to dyskaryotic cells. In some cases the smears had to be classified as Class IV." A third discussant: "Small dyskaryotic basal cells and the markedly distorted cells of obvious carcinoma are absent as a rule." And finally: "We were prevented from making a false report only because dyskaryosis was not observed in cells of the parabasal type.

There is marked agreement here. Cells of the parabasal dyskaryotic type or small dyskaryotic type do not occur in dysplasia. The reverse of this coin, of course, is that such cells do occur in "obvious carcinoma." This can only mean that these cytologists are taking the cytoplasmic-nuclear ratio into consideration. If we are all agreed that such cells do not occur in atypical epithelium but only in carcinoma in situ or invasive carcinoma, why label them with the name of parabasal dyskaryotic cells? If we are agreed that they occur in cancer why not call them cancer cells?

THE OCCURRENCE OF DYSKARYOTIC CELLS IN CARCINOMA IN SITU

A. F. ANDERSON
Edinburgh, Scotland, Great Britain

Accepting Papanicolaou's definition of Dyskaryosis as being malignant changes confined to the nucleus of a cell, I believe no one will contest that such changes are found in carcinoma in situ. Since the term denotes malignancy, however, I find little need to use it in sending out smear reports, the usual five grades being enough. If the problem is extended to what proportion of Dyskaryosis signifies invasion or non-invasion then I am on the side of those, like Papanicolaou, who believe this question cannot be answered by examining a smear alone. I acknowledge that a high degree of success attends those who do chance a forecast of non-invasion from smears with a large proportion of dyskaryotic cells, but the question of invasion is a histological one and the only deciding factor is the thoroughness of the biopsy and serial section technique, so there is no need to be a "cytological tipster," and in fact this practice can bring cytology into disrepute if the forecast is wrong. Our Edinburgh experience of the first hundred cancers discovered by cytology and unsuspected by gynaecologists, consisted of two-thirds pre-invasive and one-third with indisputable—but early—invasion. The more healthy a malignant cervix appears, the greater the dyskaryosis and greater the likelihood of its malignancy being in situ.

DISCUSSION:

J. ERNEST AYRE, Miami, Florida, U.S.A.:

Dyskaryotic cells of premalignant type are almost invariably found in cervical smears and cell scrapings from carcinoma-in-situ. They are present in moderately high concentration, usually outnumber-

ing the fully developed malignant cells. Because of the remarkable sensitivity of the cytologic method, in-situ lesions are frequently diagnosed while preclinical and discretely localized in the region of the squamo-columnar junction. Dyskaryotic cells characteristically develop at the periphery of the neoplastic lesion and cervical smears or scrapings usually show an abundance of these cellular elements. Occasional inflammatory but benign dyskaryotic cells are found too, in carcinoma-in-situ developing on the edge of an inflammatory lesion.

JEAN DE BRUX, Paris, France:

In dysplasia as well as in carcinoma in situ, the epithelium passes without transition from a stage of degeneration without passing through a "stage of function." The later the stage of degeneration occurs, the greater will be the atypia.

With irregular dysplasia one is sometimes struck by the increased number of parabasal cells with large and dark nuclei, exhibiting a decrease in nucleic acids. The cytoplasm sometimes shows cornification with an increase in the SH radicals and the di-sulfites.

In unequivocal carcinoma (of the "microcarcinoma" type of Mestwerdt) the number of abnormal immature or undifferentiated elements is seen to be much larger. The nuclei are considerably larger and the level of nucleic acids is increased. The cytoplasm only rarely shows cornification.

Carcinoma in situ is situated between the above two entities and shows the following characteristics: (a) dyskaryotic cells which show accelerated and bizarre maturation, along with cells which show hardly any maturation or which are about to mature (i.e., cells which resemble superficial, intermediate, or large parabasal cells, but the cytoplasm of which may be clearly cornified while the nuclei remain large and dark), (b) immature elements. The dyskaryotic cells showing signs of increased maturation do not permit differentiation between carcinoma in situ and dysplasia.

We consider that a distinct percentage of these abnormal cellular elements and a distinct degree of maturation should be present to assist such a diagnosis. We have attempted to differentiate dysplasia from carcinoma in situ, microcarcinomas and invasive carcinomas. We have found that the relative number of 40% or less of abnormal immature cells in the presence of dyskaryotic cells allows us to conclude that a lesion is still non-invasive. Mature invasive epidermoid carcinomas have been excluded from this discussion.

W. KENNETH CUYLER, Durham, North Carolina, U.S.A.:

It is agreed that the diagnosis of intraepithelial carcinoma is a histologic one and that adequate studies on serial, or step-serial sections often must be made on an adequate tissue specimen to establish the diagnosis. We find it necessary in the interest of the patient, however, to recognize, as accurately as our ability and the exfoliative cytologic technique permit, the in-situ lesion because: 1) there are types of dyskaryotic cells which we do not associate with non-invasive or invasive carcinomas; 2) the opinion of the cytologist may stimulate a continued search for the epithelial carcinoma in tissue already studied in part.

Serious efforts to identify, cytologically, the non-invasive carcinoma need not throw into disrepute a reputable worker. The endeavor should be encouraged. Moreover, it seems to us that two of the most important goals of cytologic studies in obstetric and gynecologic patients are to recognize with considerable accuracy the intraepithelial carcinoma and to recognize the lesion composed of atypical epithelium which has not progressed to Stage O carcinoma.

We believe that conization is the procedure of choice to provide adequate tissue for the diagnosis of intraepithelial cervical carcinoma. If all patients from our clinics whose smears contained dyskaryotic cells were subjected to cervical conization, a large percentage of these patients would be shown by careful histological studies to have but marked atypism in the cervical epithelium, or leukoplakia in a small number of patients and no demonstrable lesion of any kind in a few other patients.

LEOPOLD G. KOSS* (by invitation), New York, New York, U.S.A.:

I am in essential agreement with the thoughts of Anderson. However, I have yet to see a lesion composed cytologically of superficial, parabasal and intermediate dyskaryotic cells and without admixture of more abnormal cellular forms which would prove invasive on pathologic examination of tissues. I should add that a clean background of the smears is an additional prerequisite for this statement. Admittedly, we are conservative in our estimation of invasion on tissue sections. Extension of in situ carcinoma to endocervical glands is not considered as evidence of invasion in our institution. We have good reason to believe that if there is doubt as to whether a lesion is invasive or not, chances are that the behavior of such lesions will be that of a pre-invasive cancer. We have never seen one of these doubtful borderline invasive lesions produce a metastasis but of course this could happen, and if it did, in the experience of any of the readers, the writer would be most appreciative for information of such cases.

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EDMUND SCHUELLER, Vienna, Austria:

It is my opinion that the cytological examination offers only the possibility of making a tentative diagnosis of cancer as such. It may, however, be possible to differentiate squamous cell carcinomas from

adenocarcinomas on the basis of cellular types. However, I do not believe that one can make any statement by means of cytology regarding the extent of the lesion because invasive cervical carcinoma and the carcinoma in situ of the cervix are cytologically identical. If dyskaryotic cells are found, in addition to "cancer cells," then it means only that the carcinomatous lesion is surrounded by atypical, but non-malignant epithelium. It is true that one may find more non-carcinomatous epithelial areas on the cervix of patients with a carcinoma in situ than with an invasive cancer. However, it is impossible to make a definite diagnosis with reference to the extent of the lesion by means of cytology alone. It is the prerogative of the histopathologist to decide whether one deals with an invasive or non-invasive lesion, after he has examined the entire area of tissue in which the lesion resides. It is my opinion that the investigation of such lesions by punch-biopsies or small wedge biopsies constitutes malpractice. The only correct procedure is a conization of the cervix which contains the external os and the entire lower part of the cervical canal.

JOSE R. DEL SOL* (by invitation), Madrid, Spain:

One can only agree with the statements by Dr. Anderson, as far as routine cytology is concerned I wonder, however, if Dr. Anderson would care to discuss the academic possibility of differentiating some cases of a carcinoma in situ from an invasive lesion by means of cytology. I refer here to the excellent papers by Reagan (1), Scapier (2), Cuyler and co-workers (3), Wied (4), and Campos (5) who expressed the opinion that some non-invasive lesions may be distinguished by means of exfoliative cytology from some types of invasive carcinomas. I could go along with Dr. Anderson if he would say that there is no "specific non-invasive cancer cell" although Nieburgs (6) states there is.

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PETER STOLL, Heidelberg, Germany:

According to the histological division of epithelial changes one may find:

Normal Epithelium	:	Normal cells
Basal Cell Hyperplasia	:	Normal cells
Unquiet Epithelium	:	Dyskaryotic cells
Abnormal Epithelium	:	Dyskaryotic cells
Undifferentiated metaplastic epithelium	:	Dyskaryotic cells
Atypical Epithelium (Carcinoma in situ)	:	Dyskaryotic cells and atypical cells
Invasive Carcinoma	:	Atypical cells (with dyskaryotic cells, if the invasive carcinoma is surrounded by carcinoma in situ)

I agree entirely with Dr. Anderson if he says that the definitive diagnosis of "invasive" or "non-invasive" cannot be made by means of the cytological examination of the smear. The average percentage of dyskaryotic cells, however, is very high in carcinoma in situ, whereas there is a relatively small percentage of classical atypical cells. If there are only dyskaryotic cells present of the type which I defined in my discussion of Dr. Graham's definition of the dyskaryotic cell in this issue, then we do not perform histological examination.

I would like to refer to the data presented in the chapter in this issue entitled STATISTICAL DATA concerning the occurrence of dyskaryotic cells in cases with inflammatory reactions. Wied (1) showed previously that inflammatory reactions are less marked in cases of carcinoma in situ than in cases of invasive carcinoma. In smears of carcinoma in situ one usually observes a very "clean" cell smear which permits the differentiation of dyskaryotic and atypical cells. In cases of invasive carcinoma, however, secondary inflammatory changes are usually present.

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Wied, G. L.: Am. J. Obst. & Gyn. 71: 793-805, 1956.

GUILLERMO TERZANO, Buenos Aires, Argentina:

Both in scrapings of the cervix with Ayre's wooden spatula, and in specimens aspirated from the posterior fornix, we have found the cells we called dyskaryotic, in the smears of women who have either a carcinoma in situ or an invasive carcinoma.

The presence or absence of dyskaryotic cells in smears cannot allow of a genuine differential diagnosis between in situ or advanced carcinoma. Cells of the dyskaryotic type can be desquamated from that zone of "local reaction" around a carcinoma, a zone similar to that found in some benign lesions of the uterine cervix.

Nevertheless, dyskaryotic cells in smears, especially those of the parabasal type, must be considered as a very suspicious sign of an early carcinoma in situ.

HANS KLAUS ZINSER, Cologne, Germany:

There is no doubt that cellular findings of the dyskaryotic type will indicate the presence of an epithelial atypia which will be later demonstrated histologically as carcinoma in situ or an early invasive carcinoma. The tentative diagnosis of carcinoma in situ by means of exfoliative cytology may be made with a high degree of probability. We grade these findings Class IV according to the Papanicolaou classification and find that we have only approximately 1% false positives. Dyskaryotic cells are, however, not the only cell type which may be found in cases of carcinoma in situ. Furthermore, it should be emphasized that smears containing dyskaryotic cells may derive as well from carcinoma in situ as from an invasive lesion. At least 50% of the carcinomas in situ, which were histologically verified, exhibited a smear type which might well be observed in invasive lesions. In these cases we cannot make any prediction of the extent of the lesion. The relation between the findings in the cytological smears and the histological sections are, therefore, not definite enough to warrant an exact diagnosis.

CLOSING REMARKS:

A. F. ANDERSON:

I am much gratified to find so much agreement, Zinser's remarks especially filling out my arguments. I applaud Schueller's forthright statement that punch biopsies constitute malpractice, but I cannot follow his "atypical but non-malignant" dyskaryosis. Several others also imply a belief in such a picture, but further discussion is valueless. It is a matter of definition and I submit that we all constantly forget how subjective are our impressions. If pathologists can disagree about the identity of invasive neoplasms, then in situ neoplasms, or less than in situ as described by Cuyler, are of necessity liable to lead to much greater variety of opinions. Koss's remarks are specially helpful in explaining that the forecasting of in situ lesions is based on experience, not on principle, and I cannot believe that the surface cells are the least influenced by the first invading tongue of malignant cells into the connective tissue. We are as conservative as Koss and are steadily collecting cases of borderline invasion treated by less than radical methods. To please Del Sol let me say I believe there is no specific non-invasive cell; those who maintain there is are forgetting Kermauner and Schottlander's early work demonstrating invasive and in situ lesions side by side—but identical at the surface. We keep defining in situ lesions as identical with invasive but for the invasion. We cannot have it both ways.

One can only applaud Cuyler's attempt to recognize cancer before it has even reached an in situ degree; this discussion could go on forever. None of the discussants has persuaded me of the necessity to be a cytological tipster. What we need in this country is more clinician and pathologist acceptance of the potentialities of the in situ lesion.

* * *

OCCURRENCE OF DYSKARYOTIC CELLS IN INVASIVE CERVICAL CARCINOMA

HANS KLAUS ZINSER
Cologne, Germany

In order to discuss this problem, we have to distinguish between the early invasive carcinomatous lesions and extensively invasive carcinomas. From serial sections of tissue obtained in biopsies one may find in some cases an early invasive lesion even though the cytological smears showed only dyskaryotic cells. In more extensive infiltrating processes, however, the classical dyskaryotic cells may not be observed. The appearance of the dyskaryotic squamoid cells is characteristic of carcinoma in situ and the early invasive squamous carcinoma. The presence of dyskaryotic cells practically excludes, on the other hand, the presence of an extensive malignant process. The main value of exfoliative cytology in this particular field is to distinguish between pre-clinical (sub-clinical) lesions and clinically evident carcinomas.

DISCUSSION:

J. ERNEST AYRE, Miami, Florida, U.S.A.:

The presence of dyskaryotic cells in invasive cancer depends upon whether the cervical lesion is preclinical or is clinically involved in malignant ulceration. In the latter case where the lesion is more advanced the cytologic field is almost exclusively made up of frankly malignant cells, red blood cells, pus cells, and necrotic debris. Few dyskaryotic cells are found unless direct scrapings are taken from the epithelium at the margin of the ulcerated area (and this is an illogical or unnecessary procedure for diagnostic purposes).

In preclinical invasive cancer dyskaryotic cells are a common finding, arising at the periphery of the lesion, demonstrable readily in direct cell scrapings taken from the squamocolumnar junctional region. The discovery of numerous dyskaryotic cells, along with cancer cells, therefore provides an indication that the malignancy is likely to be early and preclinical, rather than to be in a more advanced ulcerating stage.

JEAN BERGER, Basel, Switzerland:

We are in complete agreement with Zinser's observations. However, we find fewer dyskaryotic

cells in the early invasive carcinomas which correspond to the international classification of the cervical carcinoma Stage I. If there is a highly differentiated or decayed carcinoma, one usually misses the dyskaryosis. Even when the dyskaryotic cells are not present in severe infiltrations, a histo-pathological diagnosis must be made. The presence or absence of dyskaryosis permits, at best, a finer cytological classification but never takes place of a definitive histological diagnosis. For that very reason the presence of dyskaryotic cells never permits a definitive operation but indicates a further control and clarification.

W. KENNETH CUYLER, Durham, North Carolina, U.S.A.:

Dyskaryotic cells, in our experience, are associated frequently with both non-invasive and invasive carcinomas and with both early and extensively invasive cancers. We do not find the dyskaryotic squamoid cells "characteristic" of intraepithelial carcinoma. Perhaps the unfortunate use of the word "characteristic" occurred in translation.

There may be no dyskaryotic cells in smears associated with proved intraepithelial carcinoma and there may be but dyskaryotic cells in advanced carcinoma of the cervix, and particularly in extensively invasive vulvar cancer. We associate superficial cell dyskaryosis, and intermediate cell dyskaryosis in some patients, with atypical lesions not yet intraepithelial carcinoma in extent and with certain stages of leukoplakic growth.

Parabasal cell dyskaryosis is our most dependable criterion for non-invasive carcinoma. This is true also, depending upon associated cell-types, for early invasive cancer. Intermediate cell and basal cell dyskaryosis often are associated cell-types in these lesions.

JOHN J. SULLIVAN* (by invitation), Auckland, New Zealand:

The clear concise treatment of this subject by Zinser leaves no room for disagreement.

I would, however, point out that insofar as the relative density of dyskaryotic cells becomes progressively less, when we consider their occurrence in carcinoma in situ and extensive infiltrating carcinoma, their relative or absolute absence from smears of invasive carcinoma may, in certain cases, assist in a presumptive diagnosis of invasive carcinoma when considered along with other factors (1).

It is not intended that this proposition should in any way discredit the necessity of confirmatory biopsy in all such cases.

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*John J. Sullivan, M.B., Ch.B., is the Pathologist of the National Women's Hospital, Auckland, New Zealand. Address: 8 Lovelock Avenue, Auckland, New Zealand.

GUILLERMO TERZANO, Buenos Aires, Argentina:

There are circumstances where the differentiation cytologically of carcinoma in situ from advanced carcinoma may be especially important for the prognosis of the patient. In other words, should we advise the patient to wait or have a radical operation?

Much remains to be clarified by more research so we can better handle difficult problems such as the management of carcinoma in pregnant women.

We have looked for the patterns described by Ayre (1) and by Campos (2). Up to the present, however, we may only say that the difference between advanced carcinomas and early carcinomas, is the fact that it is easier to find well-preserved cancer cells in the early cases.

As for dyskaryotic cells, we have found them in both circumstances.

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CLOSING REMARKS:

H. K. ZINSER:

The interesting discussions of my paper show general agreement on the main points.

Dyskaryotic cells may be found in invasive and non-invasive lesions. It is, therefore, not possible nor was it intended to prejudge the histological findings which will be the diagnostic procedure in every case. One may, however, find that smears containing dyskaryotic cells, especially of the deeper cell variety, will usually derive from very early lesions (non-invasive or early invasive). In smears of extensive malignant lesions one may find some dyskaryotic cells, but they will be found together with differentiated or undifferentiated "malignant" cells. My opinion is in full agreement with the very interesting statements of Cuyler. I meant to say that "nur der klassische zytologische Befund der Dyskaryose kommt bei ausgedehnten klinischen Karzinomen in der Regel nicht vor" which means in English translation that the "classical" cytological features of dyskaryosis are usually not found in patients with extensive clinical carcinomas.

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OCCURRENCE OF DYSKARYOTIC CELLS AS A RESULT OF IRRADIATION

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

In some respects the title of this discussion is misleading since in my opinion true dyskaryotic cells do not appear as a result of radiation.

The effects of radiation on benign squamous cells are four, vacuolization of the cytoplasm, increase in nuclear and cellular size, multiple nuclei, and nuclear change. The increase in size and the nuclear changes are the two changes which may be confused with a dyskaryotic cell. A dyskaryotic cell is not merely a large cell. It is a cell with adequate cytoplasm and a malignant nucleus. Because a cell is large and the nucleus is increased in size does not indicate a dyskaryotic cell unless the nucleus is abnormal. The great majority of the squamous cells exhibiting size increase have a large nucleus with even, finely divided chromatin.

Probably the large radiated cell with nuclear change is the most difficult to differentiate from the dyskaryotic cell. It is often hyperchromatic, but increase in chromatin only is not sufficient evidence of a malignant nucleus. The nucleus must show true abnormalities in chromatin distribution. The radiated cell with nuclear change does not have an abnormal chromatin pattern. Its chromatin is increased in amount but is evenly dispersed throughout the nucleus. It has one outstanding feature. A large wrinkle runs the length of the nucleus. This large wrinkle is probably because the nucleus has increased in size and a further increase in nuclear surface could only be accomplished by wrinkling in that surface. Such wrinkles should be recognized as changes in nuclear surface and not as chromatin abnormalities.

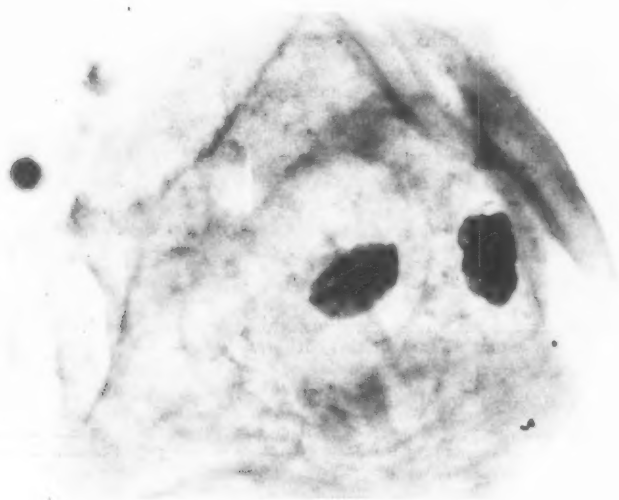

Fig. 1. Typical nuclear changes in a pre-cornified cell showing radiation change. Note that the surface of the nucleus has many wrinkles. This is not a change in chromatin distribution but rather a change in nuclear surface.

DISCUSSION:

JEAN BERGER, Basel, Switzerland:

Following x-ray and radium irradiation there appear certain nuclear alterations in normal as well as in atypical cells. These have already been described and mentioned above by Dr. Graham. One cannot consider "dyskaryotic cells" to be a consequence of irradiation. It is most difficult to make a differential diagnosis between dyskaryotic cells and cells with nuclear alterations as a result of irradiation. This is indicated by Graham as well.

We especially want to point out cytological changes following x-ray-castration with doses of 300-500 r. In these cases, within several months of the castration and often earlier, hyperchromatic, non-inflated cells displaying highly irregular nuclei are seen. Although these cells are not "dyskaryotic cells," they bear a strong resemblance to them. Here especially, because of the difficulty in differential diagnosis, only repeated examinations permit a final decision.

If "dyskaryotic cells" appear during or after irradiation (mainly in the superficial layers and with partly acidophilic cytoplasm) and the typical changes in other cells following irradiation are absent, we have an indication that either a resistence to irradiation has occurred or that a new atypical growth potential exists. Here too, one must make repeated smears to arrive at a definite conclusion.

OLLE KJELIGREN, Gothenburg, Sweden:

I agree with Dr. Graham that dyskaryotic cells should not be mistaken for cells which have been altered by irradiation. Most of the radiation changes are very typical and are easily recognized by a cytologist trained in radiation cytology. Only in cases where heavy doses of irradiation have been given to the vaginal mucosa, such as in patients treated with telegamma-rays through a vulva-portal, are there difficulties in interpretation of some cells from the basal layers. This is especially true when the typical and more spectacular part of the radiation response has disappeared. It can then be difficult to decide if one is dealing with persistent malignancy or persistent radiation changes in an atrophic smear.

GUILLERMO TERZANO, Buenos Aires, Argentina:

Dyskaryotic cells appearing in the smears of women who have received irradiation cannot be considered as "a result of irradiation" unless the cells (nuclei and cytoplasm) show the well known post-radiation changes.

We have found dyskaryosis in five irradiated women. The dyskaryotic cells in these five cases did not show any evidence of the previous irradiation, although signs of Radiation Response were observed in other cells in all five cases.

We consider dyskaryotic cells (in our cases) as the actual dyskaryotic type, described by Papanicolaou, and do not attribute their appearance to radiation.

CLOSING REMARKS:

RUTH M. GRAHAM:

These discussions emphasize one point clearly. Although everyone has agreed that dyskaryotic cells do not occur as a result of irradiation, there is also unanimous agreement that the nuclear changes produced by irradiation are sometimes difficult to interpret. This is particularly true if the cytologist is not well acquainted with radiation changes. For this reason it is suggested that if there is any question of whether one is dealing with a recurrence after radiation or with only radiation changes in benign cells, the positive diagnosis should depend on finding undifferentiated cancer cells.

* * *

WHAT IS THE PRE-CANCER CELL COMPLEX AS COMPARED WITH DYSKARYOSIS?

J. ERNEST AYRE
Miami, Florida, U.S.A.

The pre-cancer cell complex as originally defined and illustrated in 1947 (1) appears to represent the same group of cells generally included under the term "dyskaryosis." Described again and defined as "nearo-carcinoma" (4) following long-term morphologic and cell behavior study, cells of this type were described as belonging to the cancer family. Characteristic of the developing embryonic stage of neoplasm, the cells were believed to correspond to a histological stage approximating carcinoma in situ or to be approaching carcinoma in situ. Recognition of the remarkable sensitivity of the cytologic method as compared to problems of histological confirmation of a preclinical in situ cancer led to the conclusion that nearo-carcinoma represented a cytologic stage of cancer preceding the histological.

The technique used in collecting cells for diagnosis undoubtedly influences each interpreter's diagnosis as "suspicious or premalignant." Single cells in dilute secretions would be labelled "suspicious" while a surface scraping of cells from the lesion provides conclusive diagnosis of "dyskaryosis" or pre-cancer cell complex. The writer believes that some cytologists have included the borderline inflammatory or metaplastic cells showing pre-cancerous tendencies under the category of dyskaryosis. Regression of such lesions is to be expected. Further confusion arises from the accepted practice of confirming all malignant or premalignant lesions by biopsy, undoubtedly resulting not infrequently, in excision of the "heart of the lesion." Again following such biopsies, apparent but false regression may take place. The writer is convinced that true spontaneous regression of cells of the pre-cancer cell complex type is a rare phenomenon.

The final criterion of the nature of dyskaryosis or of premalignant cells depends upon ultimate behavior of the undisturbed lesion. Following an eleven-year study of such cases the writer is convinced that cells of the "pre-cancer cell complex" type represent a phase of the life history of the cancer process.

Dyskaryosis is a non-specific term indicating nuclear disturbance, and yet there is frequently cytoplasmic change as well (note: cell giantism).

It would appear, according to strict definition, that dyskaryosis includes both premalignant and inflammatory and some other atypical benign cells showing nuclear aberrations.

It seems to the writer that non-specific terms are necessary and are more widely acceptable during the introductory phase of research pertaining to a new cell-complex or cell-entity. But as time and experience reveal more factual evidence that such cells occur consistently in early infiltrating cancer, in cancer in situ, and in precursor states, gradually they should be recognized and labelled as potentially malignant or premalignant. Meanwhile, the introduction of additional terms such as "dysplasia" et al, is redundant and tends to confuse rather than clarify.

In diagnostic practice, patients showing the pre-cancer cell complex are advised to have periodic retesting at quarterly intervals or at the most to have ring-biopsy procedures, thus avoiding unwarranted radical surgery. At the same time, and of equal importance, the future welfare of the patient is safeguarded.

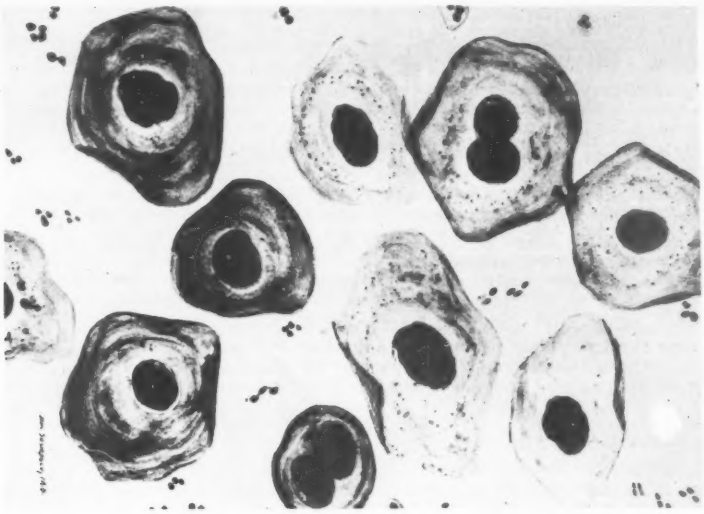

Fig. 1. Epithelial cells observed in inflammatory processes showing nuclear features diagnosed by some cytologists as "Dyskaryosis." Such cell changes frequently undergo spontaneous regression.

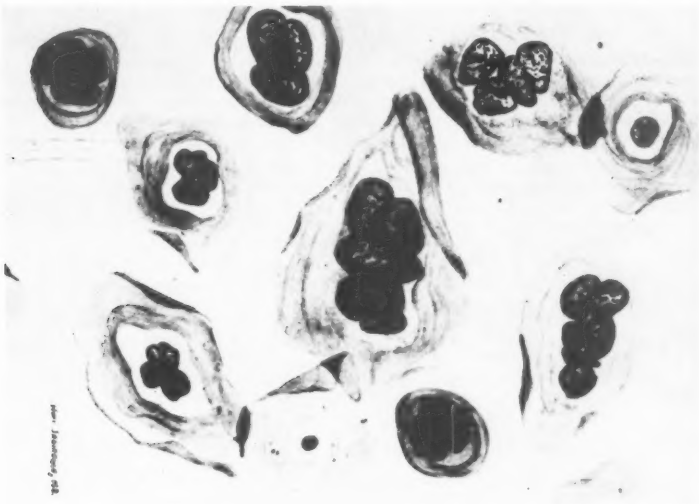

Fig. 2. Cells of "Pre-cancer Cell Complex" type. Observe nuclear changes occurring in basal, intermediate and superficial cells, frequent clearly-defined perinuclear halo. Note distinctive cell giantism with enlarged cytoplasmic envelope. Lesions exfoliating cells of this type have not been observed to undergo spontaneous regression unless disturbed by biopsy, electrocauterization, or other surgical or radio-therapeutic treatment.

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DISCUSSION:

W. KENNETH CUYLER, Durham, North Carolina, U.S.A.:

The terms pre-cancer cell complex and nearo-carcinoma apparently are concerned primarily with variants in superficial and intermediate cell dyskaryosis.

Dyskaryosis probably has come to cover a greater range of cell-types than was originally described by Papanicolaou. As a result, cells with dyskaryotic nuclei which do not appear malignant are included under dyskaryosis.

It is agreed that truly benign cell-types may be confused with what now commonly are called dyskaryoses. We believe, also, that certain cell-types (superficial and intermediate) with true dyskaryotic nuclei are associated with genital lesions which can be shown by histopathologic studies to consist of atypical epithelium, but which do not satisfy the criteria for intra-epithelial carcinoma.

By means of detailed studies and the use of a break-down classification, the kinds of cells with dyskaryosis may be segregated. Close correlation of histopathologic material, and the diagnoses made therefrom, with detailed classified cytologic findings enables one to associate in general, and quite definitely in some categories, certain dyskaryotic cell-types with certain pathologic lesions and diagnoses.

The term pre-cancer cell complex is misleading. The prefix pre is objected to because of the connotation of inevitable development of invasive cancer. Some of these dyskaryotic superficial cells may be from lesions which in time may develop into carcinoma in situ. It is probable that some of these in-situ carcinomas will develop into invasive cancer. This evolution of cancer is by no means certain. We cannot believe that all of the patients in our clinics whose smears contain superficial and intermediate cell dyskaryosis will eventually have invasive carcinoma. The incidence of cervical carcinoma would reach a figure which we now cannot conceive through logic or numbers.

It has been our contention for a number of years that the interpretation of the exfoliative cytologist should describe and identify the significant feature of genital smears whenever possible in terms used by the clinical pathologist. This would eliminate much confusion in terms and would indicate, for anyone concerned, what the cytologist would expect the pathologist to report on concommitant tissue studies. Correlative cytologic and pathologic studies permit this policy with many terms. The terms negative and positive should be discarded. Misleading terms should be relegated to disuse.

Papanicolaou's method of studying epithelial changes in the cervix is recognized as a practical one and is used instead of the biopsy because of the infeasibility of making the biopsy as routine as is the genital smear. Furthermore, it has been postulated by many physicians that the collection of cells exfoliated from the vagina, cervix and endocervix offers a better method of detection than random biopsies. This does not mean that the cytologic method should be preferred or accepted as a definitive means to establish the diagnosis of a pathologic state.

That it may require an unusually large number of histopathologic sections to locate a lesion which is associated with superficial dyskaryosis is granted. Nevertheless, the existence of a cervical lesion of this character should not be considered proved on the basis of exfoliative cytology alone. Smear constituents can vary widely, even during short periods of time.

In the study of superficial dyskaryotic cells, one must always consider the question of whether or not the true pathologic state of the cervix is indicated in the smear preparation under study. Repetition of cytologic studies may or may not settle this question. For example, in the study of 338 patients (1) who had intra-epithelial carcinoma of the cervix by histo-pathologic diagnosis, the smears from 7 patients contained no more severe changes than those of the superficial dyskaryosis defined and described elsewhere in this ACTA CYTOLOGICA. The smears from an additional 32 of these 338 patients contained superficial and intermediate cell dyskaryosis. Smears from 12 of the 338 patients did not contain any kind of dyskaryotic cell. In our work, these cells alone do not signify intra-epithelial carcinoma, although they may be prominent in smears from patients who have either carcinoma in-situ or invasive cancer. We elect to follow patients, whose smears contain the milder dyskaryotic cells (superficial and intermediate), with repeated cytologic studies. If and when parabasal or basal cell dyskaryosis appears, a cold knife conization of the cervix would be performed. It is presumed that a biopsy would have been obtained had there been present an apparent lesion.

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RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

It appears that the cells Ayre classifies as the pre-cancer cell complex are very similar if not identical to the cells I consider as dyskaryotic. Certainly dyskaryotic cells are often multinucleated. I have not been impressed by the presence of peri-nuclear vacuolization in dyskaryotic cells and am always suspicious of Trichomonas infestation when perinuclear vacuolization is common. I would like to ask Dr. Ayre to define the distinguishing characteristics between the cells he classifies as pre-cancer cell complex and those he classifies as dyskaryotic. From the photomicrographs the only distinguishing characteristic appears to be a completely pyknotic nucleus in the dyskaryotic cell.

I agree completely with Ayre that the actual potentialities of these cells, whether we call them the pre-cancer cell complex or dyskaryotic, can only be determined by following patients having such cells without definitive therapy.

LEOPOLD G. KOSS* (by invitation), New York, New York, U.S.A.:

I was very much interested in the illustrations accompanying Ayre's paper: Ayre presents two groups of cells, one of which is labelled as an "inflammatory atypia" and amenable to regression, and the other the "pre-cancer cell complex." If illustrations are to be judged at their face value, and they are frequently misleading, the cells presented as inflammatory appear to be neoplastic to this writer. At any rate they seem to present features of nuclear atypia which fall into the category of changes characterized as dyskaryosis. In fact, the changes presented here as inflammatory appear substantially more significant than those shown by Ayre in his book (1), but perhaps this difference is due primarily to the printing techniques employed here. At any rate one is thoroughly tempted to ask, "what did the biopsies show?"

I am in agreement with Ayre that the cellular changes presented as the "pre-cancer cell complex" also fall into the category of dyskaryosis. Specifically, superficial and intermediate dyskaryotic cells, especially those with perinuclear halos or cavities constitute the type of cells under discussion.

There is ample evidence that cellular changes labelled by Ayre as "pre-cancer cell complex" do exist. We have all seen them on numerous occasions and what remains is the question of origin and interpretation. Both Ayre and the writer have collected ample evidence of origin of the cells under discussion. I should like to quote Ayre's own captions to his excellent illustrations of the counterpart of the "pre-cancer cell complex" (1):

Fig. 137, p. 16	"Anaplastic tissue adjacent to early cancer."
Fig. 138, p. 163	"'Pre-cancer cell complex' Histologic findings designated as nearo-carcinoma."
Fig. 139, p. 163	"Tissue or origin of anaplastic cells of 'pre-cancer cell complex' . . . immediately adjacent to area of in-situ carcinoma."
Fig. 224, p. 245	"Biopsy adjacent to lesion" (carcinoma in-situ).

Thus, in spite of some minor inconsistencies, even Ayre has to admit that the tissue pattern, which I prefer to call koilocytotic (Koilos-cavity, kytos-cell, osis-condition) (2), cannot be referred to as cancer although on frequent occasions there is association of this pattern with cancer. This latter point is in full agreement with my own observations but to accuse the koilocytotic pattern of being precancerous in nature would be implying guilt by association.

I do not pretend to know the exact meaning of the "koilocytotic atypia." Its frequent but not inevitable association with cancer of the cervix suggests some kind of a common denominator, but to speak of "precancer" would imply that there is evidence that this tissue pattern actually changes into in-situ and eventually invasive cancer. Such proof has not been established up to the present time to the best of my knowledge. On the other hand, we observed that the koilocytotic lesion if uncomplicated by carcinoma, disappears as a rule, whereas only a small percentage of in-situ cancers cannot be traced in surgical specimens.

I wonder about those of Ayre's cases in which the pre-cancer cell complex apparently progressed to carcinoma. Could it be that a focus of cancer was present in these cervices at all times? This could not be determined with certainty unless biopsies were taken. Notwithstanding the very high degree of accuracy of cytology in experienced hands, investigation of tissue patterns still constitutes the baseline of our knowledge.

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* Leopold G. Koss, M.D. is Director of Cytology, Strang Cancer Prevention Clinic Memorial Center, and Associate Pathologist, Memorial Center for Cancer and Allied Diseases. Address: 444 East 68th Street, New York, New York, U.S.A.

J. C. de LAGUNA, Mexico, D. F., Mexico:

We agree with the author that, strictly speaking, the term dyskaryosis, as commonly used by many cytologists is too large and lacks specificity, in so far as it includes both malignant and benign alterations. In our practice, we restrict the use of such term to a picture with cytoplasmic and nuclear changes the same as Ayre's "Pre-Cancer Cell Complex" (figure No. 2). We never include the frankly benign inflammatory alterations, like the ones shown in Ayre's paper Fig. 1, although they are dyskaryotic "in senso stricto."

Histological studies of the restricted picture reveal cervicitis, hyperplasia, "dysplasia" or "anaplasia" (according to some pathologists), dyskeratosis, etc., with atypias and nuclear disorientation that are insufficient to call carcinoma in situ.

We do not know when a carcinogenic factor is operative in these cases. The fact that a lesion with this cytological picture is able to regress, and we have observed such regressions, signifies that both inflammatory lesions on the borderline of reversibility and actual precancerous lesions may have the same picture, and can be difficult to differentiate. Accordingly, we use the term "dyskaryosis" for

the whole group and talk about "Pre-Cancer Cell Complex" only in the cases in which the cytological follow-up shows a progressive Dyskaryosis, but considering that, they may still be reversible. The pictures that invariably become carcinoma in citu should be placed beyond the line of reversibility, as soon as they are discovered.

Basal hyperactivity and some metaplasias, that we consider as pre-cancerous lesions, should not be included under the term dyskaryosis since they can be easily identified and labelled by their proper names.

HERBERT E. NIEBURGS* (by invitation), New York, New York, U.S.A.:

The term "dyskaryosis" was introduced by Papanicolaou (1) to designate nuclear changes in cytologic specimens associated with epithelial lesions other than invasive carcinoma. In "carcinoma in situ" and epithelial lesions preceding this stage of cancer, the cellular changes are mainly confined to the nucleus. The suggested sub-division of dyskaryosis into: Superficial, intermediate, parabasal and endocervical, is of utmost importance if well understood by the pathologist and clinician. The cell types found in cytologic specimens reflects the stage of epithelial alteration (2). Superficial cell dyskaryosis is usually not associated with carcinoma in situ and is often seen in the presence of epithelial changes such as koilocytotic atypia described by Koss and Durfee (3). Intermediate and parabasal cell dyskaryosis are more often an indication of carcinoma in situ and not infrequently associated with early invasive carcinoma in the case of parabasal cell dyskaryosis.

Although a close correlation between the cellualr morphology and epithelial changes has been observed, the diagnosis on the basis of cytologic specimens should be limited to "suggestive." The term "pre-cancer cell complex" or "nearo-carcinoma" implies that subsequent malignant transformation is certain. As yet no convincing evidence is available that the "nearo-carcinoma," which is apparently mainly superficial cell dyskaryosis, is to be regarded as a precursor to carcinoma.

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M. JUNE SCUDAMORE* (by invitation), London, England, Great Britain:

It appears that Ayre understands the term Dyskaryosis to include:

1) Borderline inflammatory or metaplastic cells.
2) Some atypical benign cells.
3) His premalignant cells designated nearo-carcinoma.

Many cytologists find that Dyskaryosis due to infection does not confuse them, but in the absence of infection they would hesitate to say which Dyskaryotic cells indicate the presence of a lesion which would remain benign and disappear, or which would inevitably become malignant. One would expect group 3 to be the type of cell frequently seen, in addition to the definitely malignant cells, in carcinoma-in-situ. The definition (1951) to which Ayre refers, however, includes other cells usually embraced by the term Dysplasia; this he virtually admits by stating this term to be redundant. But neither "Dysplasia" nor "Dyskaryosis" are generally regarded as a "conclusive diagnosis," whereas Ayre appears to regard nearo-carcinoma as a "conclusive diagnosis."

I appreciate Ayre's objection to biopsies interfering with cytological studies; biopsy in routine practice is not indicated unless one suspects a malignant lesion on clinical or cytological grounds, and for the individual patient's welfare there is no point in making a definite diagnosis of malignancy before what is generally regarded as the in-situ and, therefore, still easily curable stage. It is therefore important to know where the dividing line is between nearo-carcinoma and actual malignancy. Ayre does not make this clear, either cytologically or pathologically; some of his cases with a "conclusive diagnosis" of nearo-carcinoma may already have actual cancer, and some may never develop it if left alone.

From the point of view of etiological studies, however, Ayre's approach has much to recommend it, and his observations may well prove correct; but as we already have much to take on faith in dealing with early malignant lesions, as well as much disagreement as to what constitutes a malignant lesion or even a Dyskaryotic cell, Ayre must do more to justify his use of the term "Pre-cancer Cell Complex" as opposed, simply, to Dyskaryosis. I should be the first to allow him more than mere photographic evidence.

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*M. June Scudamore, M.B., B.S., M.R.C.O.G., University College Hospital, London, England.

JOSE R. del SOL* (by invitation), Madrid, Spain:

The drawings of the cells identified by Ayre as "dyskaryotic" are not what I would identify as dyskaryotic. In my opinion, his cells exhibit only macronuclei. I would prefer to call these cells "dismature (e.g., a normal parabasal type nucleus in a superficial type cytoplasm), but not "dyskaryotic" which implies to me a definitely abnormal nucleus.

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The drawings of the cells which were identified by Ayre as "Pre-Cancer Cell Complex" represent in my opinion either dyskaryotic cells or rare atypia, especially the superficial cell types with multinucleation and irregular chromatin distribution. I have seen a few post-radiation cases which showed similar multinucleated cells. If the "Pre-cancer cells" were indicative of a pre-malignant lesion which would inevitably develop into malignancy then we should find practically as many patients with "pre-cancer cells" as patients with clinically unsuspected early malignant lesions.

As far as the term "pre-cancer" is concerned, I feel it is an unfortunate one. The term "pre-cancer" implies to the reader that the atypia found forecasts an inevitable course toward a future malignancy. It is my understanding that we do not know at this time of any clinical, histological, or cytological condition of the uterine cervix which will inevitably result in a malignant lesion. I do not believe the question of whether a carcinoma in situ will inevitably result in an invasive carcinoma is entirely settled, not to speak of a condition which ranges, by definition, prior to the carcinoma in situ.

I would say that neither "dyskaryosis" nor "dysplasia" is an ideal term since both show our inability to correctly identify histologically or cytologically the morphological features. The term "pre-cancer," however, does, in my opinion, go beyond the limitations of cytology and our present knowledge of carcinogenesis. Some multinucleated cells with perinuclear halo were described by Koss and Durfee (1) as "koilocytotic atypia." I wonder if Dr. Ayre would be kind enough to comment on whether or not these koilocytotic atypia are, in his opinion, identical with his pre-cancer cell complex, and, if not, by which criteria do they differ, one from the other?

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JOHN J. SULLIVAN* (by invitation), Auckland, New Zealand:

It would appear that the term "pre-cancer cell complex" (nearo-carcinoma) is used (1) to describe the atypical epithelial cells found in a surface scraping of the cervix and (2) to provide a definitive diagnosis of the lesion from which such cells derive.

With reference to the first concept the term is used somewhat synonomously with Dyskaryosis; to record the presence of abnormal cellular patterns found cytologically in atypical, precancerous and early malignant lesions. However, Papanicolaou did not propose that the presence of such cells should provide a conclusive diagnosis of Dyskaryosis.

The writer is not clear on what Ayre means when he says that "further confusion arises from the accepted practice of confirming all malignant or pre-malignant lesions by biopsy."

On the contrary, many apparent inconsistencies appear in the literature regarding the regression of lesions labelled carcinoma in situ due to the fact that a definitive diagnosis has been presumed cytologically in cases which have not been subjected to histological verification.

There is no disagreement that the "pre-cancer cell complex" may embrace "pre-cancerous lesions" but there is disagreement that the cytological method is, per se, a valid method of establishing a definitive diagnosis, when at best the diagnosis can only be a presumptive one.

The introduction of the term "dysplasia" as used by Reagan, and connoting, as it does, a variety of lesions of varying grades of severity from atypical to carcinomatous epithelium, has done much to clarify rather than confuse the true histological nature of early malignant lesions.

If it is accepted that there is a gradual aggravation of epithelial atypia preceding carcinoma in situ does Dr. Ayre suggest that such changes can be accurately and reliably diagnosed cytologically as nearo-carcinoma, and histologic examination dispensed with?

It must be conceded that the more severe grades of "pre-cancer cell complex" demand histological evaluation of the lesion, if cytology, is to retain its pre-eminent position as the most advanced technique for the detection of sub-clinical carcinoma. Undoubtedly, in less severe grades of dysplasia, or less severe grades of the "pre-cancer cell complex" there is a place for surveillance under cytologic control.

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GUILLERMO TERZANO, Buenos Aires, Argentina:

I think we see two different types of "anaplastic-feature-cells" in vaginal smears. Cells that show non-specific abnormalities and deserve a "non-specific" designation, and the abnormal cells that leave the actual impression of being malignant or "going to be malignant" should be called dyskaryotic.

An experienced cytologist should recognize them, but for various reasons a sharp differentiation cannot be made.

There are cell nuclei which suggest an early cancer that show precancerous tendencies, but do they belong to an unsuspected carcinoma or to a reversible lesion? If the cytologist sometimes hesitates between negative and positive, it is small wonder that he may also hesitate between dyskaryosis and pre-cancer.

When the cells that look suspicious are found in a smear that also displays abnormal cornification, large cells (giantism) and a peculiar halo around an abnormal nucleus, which may be lobulated,

irregular or hyperchromatic, the cytologist may dare to go further than "dyskaryosis" and give the alarm for a pre-cancerous condition.

We have never found a carcinoma in those patients we had classified as pre-cancer. Neither have we found signs of premalignancy in smears prior to a positive one. Nevertheless, when the type of cells described by the author are found in smears, we think of pre-malignancy and watch the patient carefully.

HANS KLAUS ZINSER, Cologne, Germany:

Among the cytological cell types identified as dyskaryotic there will be lesions later identified by histology as carcinoma-in-situ. A small percentage (approximately 1-2%) of our material shows histologically a lesion classified as metaplasia. One could conclude that the term "dyskaryosis" is too broad if it also includes metaplastic lesions.

Ayre, however, reports that he finds histologically not only carcinoma-in-situ but also "nearo-carcinomas," i.e., pre-cursors of the malignant lesion in cases where cells identified as "pre-cancer cell complex" were found with smears. The epithelial atypia of "nearo-carcinoma" is, however, not generally recognized by all pathologists and will often be classified as dysplasia or metaplasia. One may assume that some discrepancy between pathological and cytological diagnoses could occur if either pathologist or cytologist were reluctant to accept this new terminology. By and large, the terms "dyskaryosis" and "pre-cancer cell complex" are the same and most cytologists will agree to the above definition of dyskaryosis by Ruth Graham. The discrepancy of opinion begins with the evaluation of consequent histological examinations since pre-cursors of carcinoma in situ are not definitely known. It may well be possible that cytology will prove to be a very sensitive indicator which identifies such pre-cursors. I do not believe, however, that we have corresponding histological entities which would definitely identify a pre-cursor of a malignant lesion at this time.

I have observed in a few cases in which I performed serial sections of the uterine cervix that small infiltrating carcinomas could be detected, in patients who exhibited cell types which Ayre might classify as "pre-cancer cell complex." For these cases, it seems to me that such a cytological classification would be improper.

Because of the aforementioned reasons I am inclined to think that one should avoid the usage of the term "pre-cancer cell complex" and that it should be replaced in every case by the term "dyskaryosis" which does not convey any diagnostic prejudice. Unquestionably the term "dyskaryosis" does not describe the cytological entity fully as there are usually minor cytoplasmic changes in evidence in addition to the nuclear changes. However, the anomalies of the nucleus are most prominent, and I would suggest that we should retain this term "dyskaryosis" which has almost universal acceptance and which seems to me to be indispensable at this time.

CLOSING REMARKS:

J. ERNEST AYRE:

In reply to the discussion by Ruth M. Graham:

I agree with Ruth Graham that the cells of the "pre-cancer cell complex" exhibit dyskaryosis. However, I look upon dyskaryosis in a broader sense as including also some of the cells which we at the Cancer Institute would classify as "Inflammatory." We grade the cells from cervical scrapings as Grade 0 (Normal); Grade I (Inflammatory); Grade II (Anaplastic "pre-cancer cell complex": Nearo-carcinoma); Grade III Positive cells of cancer type. The perinuclear vacuolization in dyskaryotic cells in Trichomonas infestation is different, smaller and less well developed than the perinuclear halo seen in cervical cell scrapings in many patients showing Grade II cells. Although we have not been in the habit of using the term "dyskaryotic" it is a term which has had popular acceptance and if we were to use it, we would simply specify the cell findings as either "Inflammatory dyskaryosis" or "Premalignant dyskaryosis."

In reply to the discussion by Leopold G. Koss:

Koss raises some basic questions as to the correlation between tissue interpretations and cell interpretations. Such correlations have always been of extreme importance in the final judgment of the nature of cells but we are faced with a genuine challenge, as well as a unique opportunity to do research upon cancer cells "in embryo," utilizing hypersensitive cytologic techniques which enable the performance of direct cell scrapings from the squamocolumnar junction—the point of origin of the neoplasm. The cytologic method is so sensitive that cancer cells may be detected at a stage when only a few cells have undergone malignant transformation. Is such a lesion not cancer until it becomes demonstrable by tissue section? Perhaps so for practical diagnostic purposes, but herein lies the challenge and the opportunity to do profitable research since a considerable range of neoplastic activity may be found utilizing the cytologic method ante-dating this stage. Koss refers in his discussion to our histologic studies which have always been carefully conducted in efforts to confirm preclinical lesions as early invasive cancer or carcinoma-in-situ or anaplasia short of carcinoma-in-situ which (latter finding) we have designated as "nearo-carcinoma" (Greek: meaning "earliest stage"). It was never at any time our intention to use nearo-carcinoma to suggest that the lesion was a "near" carcinoma. "Nearo" is a perfectly good Greek suffix which it was felt might be more acceptable than the term "pre-cancer" which has always been somewhat unpopular. At the same time "nearo" implies by definition that we are dealing with cells and minute lesions representing an embryonic phase of the cancer process.

Several groups have undertaken behavior study on patients showing the precancer cell complex who have been followed over a period of years without treatment, electrocauterization or biopsy. Any surgical biopsy at this stage to be useful, will frequently remove the "heart" of the lesion and interfere with biological behavior. There is therefore, a great deal of accumulating evidence to show that cervical

malignancy develops in patients showing such cells and observed under these conditions (N. Y. Acad. Sciences 63: 1262-1269, 1956). The disappearance of the "precancer cell complex" or of cells of dyskaryotic type, or of Koss' koilocytotic lesions, following biopsy might indeed be expected in a fair proportion of cases.

Koss raises the question whether a focus of cancer was present in the cervices showing "precancer cell complex" which apparently progressed to carcinoma. If such a focus of cancer was present in those cervices at all times, it would be readily demonstrable in cell scrapings carefully prepared, repeatedly, from the squamocolumnar junction. I am in disagreement with Koss in his statement that "this could not be determined with certainty unless biopsies were taken." It is my belief that the cytologic method in the cervix provides the diagnostic procedure par excellence when the cancer is preclinical and localized. At this stage it is most arduous to accurately confirm such lesions histologically, and the histologic method shows its limitations. Quite frequently after early failure to confirm such lesions with first biopsies, serial sections of tissue blocks achieve success. I agree with Koss that the investigations of tissue patterns has constituted the baseline of our knowledge, but an excellent opportunity is provided to extend this knowledge through the judicious application of the cytologic method in exploring pure cell patterns over extended periods of time.

In reply to the discussion by M. June Scudamore:

I agree that "premalignant dyskaryotic cells" do correspond to nearo-carcinoma which we have defined as "a cytologic stage of cancer." We have stated further (above) that the cells described do belong to the cancer family. Similarly carcinoma in situ represents a tissue designation of cancer in the preinvasive stage. We are dealing with one disease. We have referred to the precancer cell complex or nearo-carcinoma as a stage of cancer which merges into carcinoma in situ, the earliest tissue designation of cancer. We agree that dyskaryosis is not generally regarded as a conclusive diagnosis for the reason that the lesion may be either benign or premalignant or actual carcinoma in situ, (according to some tissue interpretors). When we report nearo-carcinoma as a "conclusive diagnosis" we are expressing a diagnostic opinion based upon our best experience and judgement to date.

As to the dividing line between nearo-carcinoma and actual malignancy I would express the opinion that the former (nearo-carcinoma) is simply an early stage of the latter (malignancy). (As an eminent pathologist expressed it—when a colt becomes a horse!) Presumably nearo-carcinoma merges with carcinoma in situ just as glandular extension precedes early infiltration of stroma by malignant cells. While agreeing that cases with a conclusive diagnosis of nearo-carcinoma do already have cytologic cancer, on the other hand I am uncertain whether some may never develop malignancy if left alone, except in so far as such patients may subsequently die of intercurrent disease or lose their uterus because of a fibroid.

Cytology provides one of the very useful tools which should enable us as physicians to avoid "taking on faith" (as Scudamore expresses it) statements pertaining to early malignant lesions. While we have been collecting photographic evidence of the intriguing and colorful cellular manifestations seen in our microscopes for a decade now it does seem that both interest and understanding of these cellular phenomena is increasing. It may well be that quantitative cytochemical techniques and methods (Caspersson) give information on the chemical differences and similarities between normal, premalignant and cancer cells in the cervix, providing a long-sought answer to this important basic problem.

In reply to the discussion by Jose R. del Sol:

In reply to del Sol I cannot but agree that the term "precancer" is an unfortunate one. I think it should be pointed out, however, that from the first crude and rather inadequate description and illustration of atypical cells of this type made by our group in 1947 we defined a "cell complex," not a single type of cell, as precancerous. Our implication was that when a combination of cells of the types portrayed occurred in cervical cell scrapings we believed that we were dealing with a combination of abnormal and pathological cells indicative of a definite premalignant lesion of the cervix. Now after a decade there seems to be more evidence to justify the diagnosis of a "precancer cell complex" than ever before. However, it is perfectly understandable that others prefer the use of the cell designation "dyskaryosis." None of the many terms introduced to describe cells characterizing this developmental phase of neoplastic change seems ideal. I am inclined to agree with del Sol that the term "precancer cell" goes beyond the limitations of our present knowledge of carcinogenesis. I am inclined to think, and apparently Koss agrees, that the cells with perinuclear halo described by Koss and Durfee as "koilocytotic atypia" represent cells of similar type and character. An important difference between the behavior studies of Koss and my own is the surgical biopsy. Could this be the reason why the koilocytotic lesion "if uncomplicated by carcinoma, disappears as a rule?"

In reply to the discussion by John J. Sullivan:

Sullivan states that the meaning is not clear in the statement that "further confusion arises from the accepted practice of confirming all malignant or premalignant lesions by biopsy." The implication here was that in dealing with preclinical lesions, either malignant or premalignant, diagnosis is initiated by cytologic studies but confirmation by histology is desirable. If the "heart" of the lesion is found by the biopsy, successful immediate confirmation of the disease results. But the subsequent disappearance of the residual lesion has been termed "spontaneous regression," leading to an erroneous conclusion that the lesion was not cancer in the first place since it disappeared without radium or hysterectomy. We believe that a surgical biopsy of the embryo cancer will not infrequently represent extirpation of the lesion and apparent cure (at least pro-tem since no one knows whether new seeds of cancer cells may germinate in the same soil years later). In my limited experience I have not seen, nor have I seen reports in the literature of spontaneous regressions of lesions labelled "carcinoma-in-situ" cytologically in which histological verification was not sought. This is the type of evidence we have been seeking unsuccessfully for years. On the other hand reports of regression of such lesions diagnosed cytologically and confirmed histologically to be followed by "apparent" regression are not uncommon.

On the question of disagreement that the cytological method "per se" is a valid method of establishing a definitive diagnosis rather than a presumptive one in "precancerous" lesions, my opinion is that when we are dealing with a clinical lesion which may be cancer, cytodiagnosis must be confirmed by

histological studies to determine the extent of the growth preceding therapy. Under these conditions only the histological method provides a definitive diagnosis. In diagnostic practice it is our routine to recommend histological confirmation in all patients with positive cell findings. But when we are dealing with preclinical or previsual cancer in the cervix, the cervical cytologic method is both highly effective and remarkably simple in establishing a definitive diagnosis. On the other hand, it is only with extreme difficulty and meticulous care and after multiple or serial sections from a "ring-biopsy" that one can accurately confirm the nature of the lesion histologically. The gynecologist of future years will accept a simpler less arduous and time-consuming method of diagnosis of preclinical cancer than "ring-biopsy" and serial sections as a preliminary to curative surgical treatment. At present we recommend "ring-biopsy" which was described first by our group in 1948. But we look upon it as a stop-gap procedure which will be eliminated in most cases as cytodiagnosis becomes better standardized.

In routine diagnostic practice in the Miami Cytology Center the graded epithelial atypia preceding carcinoma in situ referred to by Sullivan, are indeed observed and we believe accurately and reliably diagnosed cytologically. We hold back recommending attempted histological confirmation until cytology indicates that the approximate "in situ" stage is reached. Even with ring biopsies widely used by the practicing medical profession, it is (as in Miami) often necessary to search tissue blocks repeatedly to confirm the lesions recognizable cytologically as carcinoma in situ. There is such a variation in diagnostic criteria as well as in histological techniques and methods, and unfortunately so often too few sections are cut! We definitely believe that direct cervical scrapings carefully studied provide accurate and reliable evaluation of precancerous anaplasia which has not yet advanced to the carcinoma in situ stage.

I heartily concur with Sullivan's implication that in routine gynecological practice, if cytology is to retain its pre-eminent position as the most advanced technique for the detection of sub-clinical carcinoma, both cytology and meticulous histological studies are necessary and they should complement one another in achieving maximal efficiency in gynecological cancer control. In the less severe grades of the precancer cell complex, we agree that there is a very definite place for surveillance under cytologic control.

In reply to the discussion by Hans Klaus Zinser:

Zinser states that "nearo-carcinoma is not generally recognized by all pathologists and will often be classified as dysplasia or metaplasia." Others may report basal cell hyperplasia or anaplasia. It is to be expected that there will be some differences in terminology between pathologist and cytologist. It is not many years since pathologists believed carcinoma-in-situ to be a rare entity. And there was great confusion concerning diagnostic criteria. The increasing use of cervical cytology has provided pathologists all over the world with early malignant lesions in great numbers. Histological techniques and methods have had to be greatly improved to keep up with the diagnostic accuracy of cytology. It is not improbable that new descriptive histological terms may be introduced as knowledge of cancer-precursor tissue changes increases. We are convinced that cytology is an extremely sensitive indicator to identify the precursor state referred to by Zinser.

Since we are dealing with one disease, whether diagnosed by the cell method or by tissue, one stage is going to merge with another. So it is inevitable that some cases diagnosed as "maximal precancer cell complex" will be interpreted by one pathologist as "carcinoma-in-situ" when the tissue is studied. On the other hand, some pathologists disagree and may use some alternative tissue designation. In other cases, the pathologist may cut too few sections and find lesser degrees of tissue atypia of anaplasia. In the cases referred to by Zinser in which small infiltrating carcinomas were detected "in patients who exhibited cell types which Ayre might classify as precancer cell complex"—in such cases I agree with Zinser that a cytologic classification as "precancer cell complex" would be improper. On the other hand, it is quite a common finding to observe the precancer cell complex associated with fully active cancer cells in extremely early preclinical lesions when cell scrapings are examined. Indeed such cell findings will frequently identify the lesion cytologically as being definitely "early" (both clinically and by histological study).

Zinser is, of course, expressing his own personal preference in the use of the term "dyskaryosis" rather than "precancer cell complex" and he has a perfect right to do so even though he admits minor cytoplasmic changes to be in evidence in addition to the nuclear changes.

* * *

DYSKARYOTIC CELLS IN PERICIOUS ANEMIA

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

In a patient with pernicious anemia in relapse, as many as ten per cent of squamous cells from the upper gastro-intestinal tract may be dyskaryotic. These cells are morphologically identical with those cells in the vaginal smear of patients with abnormal epithelium in the cervix. After the hemoglobin and red blood count have risen to adequate levels after either liver or B_{12} therapy, these cells completely disappear. Such cellular changes can be seen in the vaginal smears of young mice of B_{12} deficient mothers.

This may be an important observation since these cells are morphologically identical to the dyskaryotic cell under discussion yet they disappear when the B_{12} deficiency is corrected. This is an example of marked morphologic abnormalities being reversible.

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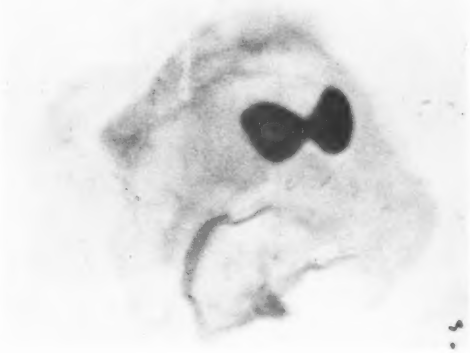

Fig. 1. A superficial cell with a malignant appearing bi-lobed nucleus. Such dyskaryotic cells as this were found in the vaginal secretion of mice of B_{12} deficient mothers. When B_{12} is restored the changes disappear completely.

DISCUSSION:

CYRUS E. RUBIN* (by invitation), Seattle, Washington, U.S.A.:

Dr. Graham's observations of squamous macrocytosis have been confirmed in this laboratory. It presumably originates in the mouth, pharynx, and esophagus during B_{12} deficiency. Similar observations have been made in the laboratory during folic acid deficiency. The cellular changes which accompany the latter deficiency also revert with treatment. Evidence is accumulating in both cytologic and histologic material which indicates that varied nutritional deficiencies may lead to macrocytosis and other cellular abnormalities in epithelial tissues with rapid turnover times. Two fascinating questions remain: 1) Are these epithelial changes ever irreversible? 2) Can they predispose to carcinoma? Present studies indicate that the answer to both questions may prove to be yes.

*Cyrus E. Rubin, M.D., is Assistant Professor of Medicine, School of Medicine, University of Washington, Seattle, Washington.

CLOSING REMARKS:

RUTH M. GRAHAM:

No comment.

* * *

STATISTICAL DATA ON DYSKARYOTIC CELLS

GUILLERMO TERZANO
Buenos Aires, Argentina

Out of a total number of 5.644 vaginal smears evaluated, dyskaryotic cells were found in 354 cases, i.e., 6.3%:

	Number of cases	Superficial type	Intermed. type	Parabasal type	Endocervical type	Endometrial type
Carcinoma of the cervix	68	10	18	37	3	-
Carcinoma of the endometrium	12	-	-	-	-	12
During estrogen therapy	3	-	-	-	-	3
Cervicitis	116	40	72	-	4	-
Premenopausal colpitis	26	9	17	-	-	-
Postmenopausal colpitis	32	5	19	8	-	-
Trichomonas vaginalis inf.	10	6	4	-	-	-
Pregnancy	22	15	6	-	1	-
Post-Radiation case	5	3	1	1	-	-
After cauterization of cervix	12	7	5	-	-	-
After D & C	6	-	-	-	-	6
Granuloma	11	4	6	1	-	-
Miscellaneous	31	15	10	5	1	-
Total:	354	114 (32.2%)	158 (47.7%)	52 (14.7%)	9 (2.6%)	21 (5.9%)

Out of the total number of 5644 vaginal smears examined we found dyskaryotic cells in the following percentage:

Superficial type	2.2%
Intermediate type	2.8%
Parabasal	0.9%
Endocervical type	0.15%
Endometrial type	0.37%

STATISTICAL DATA ON DYSKARYOTIC CELLS

PETER STOLL
Heidelberg, Germany

Of 5619 patients examined, there were 425 cases, i.e., 7.6% in which Class III reports were given.

In these 425 cases, the final diagnoses were as follows:

Corporal carcinoma	37
Cervical carcinoma	76
Dyskaryoses after irradiation	58
Primary dyskaryoses	174
Dyskaryoses with additional atypical cells (Ca in situ histologically)	24
Secondary inflammatory reaction	56

total dyskaryoses: 198
(of all examinations: 3.5%
of all Class III reports: 46.0%)

Not infrequently, we found dyskaryoses associated with Trichomonas Vaginalis infestation and Monilia albicans.

(The above papers by Terzano and Stoll were not discussed. Members of the Academy who wish to discuss these papers may send their discussions to the editorial office. These contributions will be published in the next issue of the ACTA CYTOLOGICA in the column "Letters to the Editor" - Ed.)

Symposium B

EXPERIENCES WITH VARIOUS METHODS OF FIXATION OF SMEARS

DO CELLULAR CHANGES OCCUR AS A RESULT OF AIR DRYING OF SMEARS?

J. PAUL PUNDEL
Luxembourg, Luxembourg

The air drying of vaginal smears before fixation can produce cytological changes of three types:

1. The first and most rapid change takes place in the cytoplasm of the squamous cells. Air drying before using the classic staining technique of Papanicolaou or Shorr transforms the normal basophilia to a more or less complete eosinophilia depending on the degree of drying. The etiological mechanism of this cytoplasmic change is believed to be oxidation by the air oxygen (6). Supporting evidence: The same complete eosinophilia can be produced experimentally by fixing the smears in solutions with high oxidation power like chromic acid or the Helly fixative (6). The eosinophilia by drying in air can be made reversible by rinsing the slides in a reducing solution like the Ruge solution containing formaldehyde (1) or hydrochloric acid (4). Acetic acid to a certain degree also can bring back the normal basophilia (5, 11). This cytoplasmic change which we call pseudo-eosinophilia by air drying can be the cause of error in hormonal diagnosis.

This is only a part of the problem of the cytoplasmic affinity for differential stains. The complete etiological mechanism of the physiological basophilia and eosinophilia is not yet known. Basophilia can be changed to eosinophilia artificially by changing the pH (12), using chemical agents such as isopropylic alcohol (3) or oxidizing agents (6) and the normal eosinophilia to a more or less complete basophilia by strong reducers (6), by change of the pH of the fixative (12). It is interesting that by changing the complementary stains in a differential technique the same stain can colour in one formula the basophilic cells and in another only the eosinophilic cells (7).

2. The second type of change occurs in the cytoplasm and the nucleus, but only after complete air drying. Changes take place in the morphology, form and fine structure of the nucleus and the cytoplasm. They are partly due to dehydration, but also to modifications of the chemical composition of the liquid medium of the vaginal content. These changes in general are irreversible and can not be cleared up by rehydration at a later fixation (2, 8). Air drying can be a satisfying fixation in blood smears, but not in vaginal smears, because blood smears are very thin, made of a constant and quick drying medium permitting good staining by special techniques. But in vaginal smears, which are of various media and different degrees of pH, of various thickness and variable drying degree, air drying can not be used for correct fixation. The changes which can occur are important in cancer diagnosis and can not be completely recovered by the usual staining techniques. Most cytologists believe now that in cytology as well as in histology for cancer, correct and immediate fixation of the cells or tissues is one of the essential conditions for a later correct diagnosis.

3. A third type of change, autolysis, can occur in air dried smears if the slides remain for a certain time in humid air. Modifications appear of the post-mortem type, and if the air is humid and warm, moisture can develop and destroy the cells. These changes are the same as one can see histologically in the tissues after postmortem decomposition, i.e., degenerative lysis of the cytoplasm and nucleus (11).

As these different changes can be the cause of diagnostic errors or can render the smears completely useless, it seems that all staining techniques using air dried smears should be avoided, especially in routine screening for cancer. (We are not discussing the fixation in other staining techniques for research purposes.)

The study of the etiological mechanism of the physiological color affinity of the vaginal cells remains an interesting research problem.

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CLARICE DO AMARAL FERREIRA
Rio de Janeiro, Brazil

Lichtwitz and co-workers (3) in 1949 stated that air drying is an adequate fixative method that does not change the cellular lipoprotein complex. Cellular oxidation, however, does not permit staining by Papanicolaou's technique, as in this reducing substances are used before staining. Lencioni and co-workers (2) observed also that in air-dried smears the cells do not show changes in the morphology or staining affinities for many days. Sagi and MacKenzie (4) allowed the smears to dry after fixation in acetone and slides similar to those fixed immediately in alcohol-ether were obtained.

Changes from customary techniques are usually made for convenience. For many years, therefore, because of the inconvenience of conveying many receptacles with slides in alcohol-ether from my private office to the laboratory, I have carried the unfixed slides in little boxes, and the smears were fixed in alcohol-ether in the laboratory sometime the next day. I have studied smears from the same patient, fixing one immediately, and allowing another to dry before fixing. I have not found artifacts that would invalidate the process of air drying. Sometimes the cells appear more acidophilic, but there is no modification in their morphology and cancer diagnosis is not interfered with at all. I should like to point out that we use the Trichrome stain of Shorr (5). Daily, I have the opportunity of comparing the two methods mentioned, as all smears of hospital patients are fixed immediately without drying, but the smears from my private patients and from those of some of my colleagues are fixed after air drying, and I find no significant differences, especially with reference to cancer diagnosis.

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HANNAH PETERS
Bombay, India

The changes that are seen in smears that are air dried are mainly twofold: 1) changes in the staining property of the cell and 2) changes in the appearance of the cell.

1. Cells on an air dried smear, when stained with the ordinary Papanicolaou technique, will stain pink simulating acidophilic cells. A smear that is fixed in too little alcohol and ether, so that half of the smear dries in the air while the other half is properly fixed, will show all cells on the air dried side uniformly pink while the cells on the alcohol-ether fixed side stain with a variety of blue and pink and orange according to the type of cell. The nuclei of air dried cells stain poorly; they appear pale blue and uniform; no details can be recognized.

2. The form and shape of cells are altered by drying. They swell and become bigger than they are in the fixed state. The cell outlines become indistinct and fuzzy. The nuclei undergo the same changes: their size is deceptive and might lead to a mistaken impression.

Summary:

Cells on an air dried smear usually stain pink and might therefore simulate an acidophilia. The large nuclei of air dried, swollen cells should be recognized as an artefact and not be mistaken for an abnormality.

DISCUSSION:

J. PAUL PUNDEL, Luxembourg, Luxembourg:

I, along with the majority of European cytologists, am unable to support the conclusions of Ferreira for the following reasons: Air drying of smears before fixation in alcohol-ether and staining after Shorr always gives useless slides. One can see these differences when slides are fixed immediately in alcohol-ether, but in jars containing not sufficient fixative to cover the entire smear. These slides show very clearly the difference existing between the correct fixed lower part and the upper part where air drying occurred prior to fixation of the cells. Research done by Ferin and Demol (1) which I am able to confirm, show that if we adopt as a standard for cellular morphology the appearance of the fresh vaginal content examined under phase contrast, then only the Papanicolaou or the combined hematoxyline-Shorr technique after immediate fixation give a similar cell picture with all nuclear and cytoplasmic details. Techniques utilizing air dried smears even with the classical Shorr single stain technique do not permit such a clear demonstration of the morphological details, especially of the nucleus; hence, these techniques should not be used for cancer diagnosis. It is possible that the differences between the findings of Dr. Amaral Ferreira and my own can be explained by the use of different staining formulae. I use with best results Shorr's differential stain prepared by Mahady Co., Boston, and the zinc sulfate hematoxyline of Papamiltiades (2). To clear up these differences, I propose the exchange of slides.

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JEAN DE BRUX, Paris, France:

We believe that we can recommend the use of a moistening agent for those smears which have been mailed without a glycerin covered slide.

Smears which are fixed initially in ether-alcohol for at least 10 minutes and which are received dry are immersed in a 10% solution of Lauryl-Sulfate of Sodium for 4 to 5 minutes, and then are refixed in ether-alcohol. The ordinary routine staining of the Papanicolaou technique is then followed.

By and large, a smear thus rehydrated and stained can be used for both hormonal and cancer readings.

JACQUES FERIN* (by invitation), Louvain, Belgium:

Smears were taken in the same subject during three menstrual cycles. They were air dried and colored with hemateine-eosine, fixed in alcohol-ether, and colored using the classic staining techniques of Papanicolaou or Harris-Shorr. In addition, wet control-smears were examined with a phase contrast microscope.

From this comparative study it was evident that in the air dried smears the nuclear structure was very well preserved. In addition, we could observe that at the height of the follicular phase the air dried smears showed relatively more of the typically "flat cells" than the alcohol-ether fixed smear which displayed a greater percentage of cells with early curling.

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HERBERT E. NIEBURGS* (by invitation), New York, New York, U.S.A.

Drying of cellular material in air results in the flattening of the cells with an increase in nuclear and cellular size. If no rehydration with subsequent fixation is carried out before staining, the dried cells have a tendency to turn eosinophilic and in case of cornified cells, the breaking up of the keratinized cytoplasm on drying results in the appearance of small canaliculi. The nuclear chromatin pattern often cannot be recognized following staining of previously dried cells. In the case of cervical specimens, however, a dried smear containing abnormal cells should not be missed and might even be accurately interpreted by an experienced cytopathologist. Careful consideration should be given to this fact, particularly in case of mass screening procedures.

All effects of drying except perhaps the canalization of keratinized cytoplasm can be prevented in cervical smears by adequate rehydration in water to which a surface active agent has been added (1, 2). Subsequent fixation of the material is necessary before staining. In the case of cytologic specimens other than cervical and vaginal, air drying may be permitted following immediate fixation with ether-alcohol alone or with a mixture of ether-alcohol and hydroxypropyl methyl cellulose ether (1) or diaphane (3). Both leave a hard protective film over the cellular material following drying which can be adequately dissolved in the laboratory before staining.

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EDMUND SCHUELLER, Vienna, Austria:

The air drying of cells results in marked structural changes which render an exact evaluation of smears impossible. The cytoplasm has a dirty reddish appearance and its transparency is impaired. The more important structural alterations are found in the nuclei which swell, exhibit indefinite nuclear outlines and chromatolysis. The air-drying of regularly fixed smears results also in a decrease of the quality of staining of the cells, but by no means as bad as that seen with unfixed material.

Unfixed smears which have been permitted to dry for only short time may be treated in the following way to restore cellular detail: 1) tap water, 2) 30% alcohol, 3) 70% alcohol, 4) 2% acetic acid in 70% alcohol. The smears are treated in each of the above solutions for several minutes and then stained in the usual way.

For mailing purposes, I recommend for use on the smears artificial resins, such as Diatex or Diaphone. These substances form a fine film upon evaporation and inhibit the drying of the specimens. The artificial resins are easily dissolved in alcohol-ether; the results of staining smears so treated are excellent.

PETER STOLL, Heidelberg, Germany:

I agree completely with the conclusions drawn by Dr. Pundel, and would also refer to the presentation by Dr. Ebner at the symposium on Applied Cytology in Brussels, July 7, 1957.

My own experiences are restricted to:

(1) Smears prepared in our own department and outpatient clinic which are fixed immediately in alcohol-ether and which are stained without drying after fixation, and

(2) Smears from patients of outside physicians. These smears are fixed in alcohol-ether for at least 20 minutes and are mailed to us either dry or covered by another slide with some glycerin in between these slides.

Both methods yield equally good results.

GUILLERMO TERZANO, Buenos Aires, Argentina:

By a process of re-hydration through acetic acid-alcohols the normal pH of dried smears can be restored for endocrinological evaluation. This permits once again differentiation between acidophilic and basophilic cells, and no remarkable differences can be detected when these smears are compared with smears from the same specimen fixed initially in ether-alcohol.

The avoidance of air-drying of the smears, becomes more important in cancer diagnosis. Apart from poor preservation of cells, shrinking or swelling of the nuclei, distortion, etc., may lead to an incorrect diagnosis. This particular artefact is noticeable in cells of the endocervix and the endometrium.

CLOSING REMARKS:

J. PAUL PUNDEL:

From the discussions I get the impression that there exists general agreement about two points:

1. Air drying of smears before fixation can produce some morphologic and tinctorial changes in the vaginal and cervical cells which can be the cause of diagnostic errors or difficulties, especially in cancer diagnosis. Only Ferreira is of a different opinion. As I am familiar with the careful cytological work of Ferreira, I think it would be interesting to look further for the reason for these differences between the findings of the discussants apparently using the same staining technique. For my conclusion I must say that I have never obtained satisfactory smears after air drying, even after rehydration by distilled water. The statement of Ferin, who is the pioneer of European hormonal cytology, is very interesting, but I believe that his conclusion is valuable only for his eosin-hematoxyline method and cannot be transposed to the usual differential staining techniques. For general teaching purposes, I still would recommend, especially for routine smears for hormonal or cancer diagnosis, that the smears be immediately and correctly fixed before staining.

2. If continuous storage of the smears in the liquid fixative is impossible for practical purposes, as for mailing, the agreement seems to be general, that the smears should be fixed as usual, at least for 10 minutes. After this first fixation, they can be allowed to dry for mailing, but the staining should be preceded by a rehydration with moistening agents and a second fixation. To obtain a better differentiation, acetic acid should be added to the second fixation or to the alcohols. It would be better in every case to use the glycerine technique for mailing, thus avoiding air drying, or to cover the smear with a protective resin film. I still believe that all these techniques should be used for special cases only, as necessary and not for routine practice.

CLARICE DO AMARAL FERREIRA:

I have read with great interest the opinions of my colleagues on the subject under discussion. We are in the process of making a comparative study of the cellular changes in the following three types of smears: 1) smears fixed in alcohol-ether prior to air drying, 2) smears fixed after one hour, and 3) smears fixed after 24 hours of air drying.

Air drying usually causes a pseudo-acidophilia that can be differentiated, however, from true acidophilia. I should like to point out that there are no morphologic changes in the nucleus which would disqualify the use of air dried smears for correct cancer diagnosis. I have never failed to diagnose cancer because of dried material. In this respect I agree with the conclusions of Ferin that "in the air dried smears the nuclear structure was very well preserved."

I should like to call attention to the fact that I do not recommend air drying of smear prior to fixation as routine procedure or as superior to immediate fixation. However, it is sometimes more convenient and for this reason I have been able to gain experience in this method. Further I never used the single differential stain of Shorr; I tried this once and found it unsatisfactory. I use the classical Trichrome stain with Harris hematoxyline.

I hope to be able to contribute the results of the above comparative study to the chapter "Letters to the Editor" in the next issue of the ACTA CYTOLOGICA, and I shall also be pleased to forward slides to my friend Pundel.

HANNAH PETERS:

Summarizing the discussion:

It is the opinion of most of the discussants that air drying will cause cellular changes. The cytologic changes are more marked in smears which are dried before fixation than in those dried after having been fixed for some time in alcohol and ether. The changes involve the staining quality of the cell as well as the structure of the cytoplasm and the nucleus. Through rehydration some of the changes are reversible and the smear can be used for endocriniological interpretation; however, at present, it remains doubtful whether the slides become good enough for cancer screening. The interpretation of single cells depends on their optimum preservation and staining detail which is best achieved by adequate fixation and avoidance of drying.

* * *

ADVANTAGES AND DISADVANTAGES OF FIXATION METHODS OTHER THAN ALCOHOL-ETHER

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

The disadvantage of the alcohol-ether method of fixation is that the solution is inflammable. In my laboratory we had an ether explosion when the switch of a hot plate was turned on at the same time the fixative was being filtered. After this accident we ran an experiment to see if the ether was actually necessary in the fixative. Half of the slides were fixed in ether-alcohol, half in 95% alcohol alone. The technicians screening the slides were asked to state which fixative was used. Their accuracy in doing so was that of chance. For the past three years we have used only 95% alcohol and the results of fixation have been entirely satisfactory.

PETER STOLL
Heidelberg, Germany

The fixation of smears by means of alcohol-ether or by means of alcohol alone yields practically identical results. We are currently investigating the efficacy of a mixture of acetone and water (90 to 10) and permitting the smears to dry out after fixation. This procedure would make the mailing of the smears easier. The results seem to be good.

J. PAUL PUNDEL
Luxembourg, Luxembourg

The fixation by alcohol-ether gives excellent results, provided that the mixture is fresh and the ingredients are of the best quality. Evaporation, exposure to sun light or use of impure alcohol or ether may produce changes of pH which can fall to 5.0 and result in modifications of the eosinophilic index. On the other hand, ethyl-alcohol in some countries such as Belgium and Luxembourg even for laboratory use is very expensive due to the high State taxes. I use, with as much success, the following fixation mixture: Isopropylic alcohol (absolute) 97.5 parts, glacial acetic acid 2.5 parts. This fixation method has the advantage that it can be used for a long time, that it is cheap and that it will clear the slides of red blood cells.

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DISCUSSION:

J. C. de LAGUNA, Mexico, D. F., Mexico:

We have been using routinely, for the past year, 95% alcohol as a fixative instead of the alcohol-ether mixture, after Ruth Graham told us about her experiences with it. We were able to confirm that there is no difference in the morphological characteristics of the cells. We have not checked, in our slides, for the alleged eosinophilia, reported by J. R. Pierce (Am. J. Obstet. Gynec. 74: 119, 1957) in smears that are left for some time in 95% alcohol. We think that this point deserves further clarification.

LUIS MONTALVO RUIZ, Madrid, Spain:

The advantages and disadvantages of the fixation methods are conditioned by the staining method that is going to be used. We cannot fix a preparation that is to be stained with silver in the same way as a specimen to be stained by the Papanicolaou method. In the first case, it is better to fix with formaldehyde 10%, and in the second case, it is more advantageous to fix with alcohol-ether. We are not aware of any

inconvenience with fixation using only 95% alcohol, and for us this method is cheaper, for the same reason that ether and acetone are more expensive than alcohol, used by Stoll.

We always fix routinely with alcohol-ether when using the Papanicolaou staining technique taking care not to light any matches or Bunsen burners in the laboratory where ether is being used.

GUILLERMO TERZANO, Buenos Aires, Argentina:

We utilize alcohol-ether for fixation of the smears; first, because we have had no troubles and no accidents to regret (so far), and, second, because we wish to follow as closely as possible the directions given by Papanicolaou.

In some instances, when alcohol-ether fixative was unavailable, we have fixed the smears in 95% alcohol alone. No differences were found in the stained smears.

For mailing, Ayre's glycerin technique, after one hour in alcohol-ether, has proved to be excellent.

According to Ayre (1), we are trying the mixture, "alcohol-ether-glycerin," which seems to be acceptable to those who desire easier procedures.

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CLOSING REMARKS:

RUTH M. GRAHAM:

It appears from the discussions that 95% alcohol alone gives as adequate fixation as the ether alcohol mixture. I think this is an important point since the addition of ethyl ether to the fixative is both hazardous and expensive. Furthermore, it is extremely difficult to keep the concentration of the ether at 50% since evaporation takes place so quickly.

I have had no experience with the use of isopropyl alcohol as advocated by Pundel. Those of us who have quantities of tax-free alcohol available tend to forget how expensive alcohol is if the tax is included in the cost. I intend to try this method of fixation. The addition of glacial acetic acid would seem to be necessary only in particular cases. I would hesitate to add it to the fixative routinely since I would imagine it would interfere with the quality of the stain.

PETER STOLL: No comment.

J. PAUL PUNDEL:

I can confirm that ethyl-alcohol of 95 or 96% is practically as good as the classical alcohol-ether mixture, providing the alcohol is of excellent, chemically pure quality. Unfortunately, as I have said before, this alcohol is very expensive in my country. There exist important differences in the pH of the usual industrial alcohols. We also know that variations of the pH of the fixative can produce changes in the eosinophilia of the cells (Vokaer, R.: Bruxelles Med. 33: 2471, 1953). A change of the alcohol pH could possible be the cause of the abnormal eosinophilia reported by J. R. Pierce, as quoted by Laguna.

* * *

FIXATION TECHNIQUE FOR STAINING PROCEDURES OTHER THAN THE ROUTINE PAPANICOLAOU PROCEDURE

LUIS MONTALVO RUIZ
Madrid, Spain

Disregarding Papanicolaou's method of fixation and staining, alternative fixation techniques vary when specific staining methods are used. In 1941, we initiated, following the recommendations of Professor Botella (1) our studies on vaginal cytology and took the smears with a platinum handle, fixing them in alcohol-ether for ten minutes. The staining was done in hematoxylin-eosin.

The alcohol-ether fixation in this technique was quite satisfactory but the staining procedure was soon replaced by the method of Shorr and Papanicolaou.

At that time we investigated glycogen content with Best's carmine after fixation in 96% alcohol for ten minutes. Fixation in alcohol only was satisfactory.

In 1944 we adopted Shorr's method, fixing the smear in equal parts of alcohol and ether for three minutes.

In 1948 appreciating the work done by Yue et al (7) we started to use Rio Ortega's silver carbonate, the results of which have been published (6).

Fixation of the smears in ten per cent formalin for at least fifteen minutes proved perfect.

In the last year we have investigated the histochemical features of vaginal cytology and consequently have stained the smears in alkaline glycerophosphatase. Gomori's method with Schiff's periodic acid (P.A.S.) according to Hotchkiss (4) and McManus's techniques (5) modified by Ebner (3).

For phosphatase staining the smears were fixed in 96% alcohol for one hour. For P.A.S. staining the smears were fixed in a mixture of 96% alcohol and neutral formalin as follows:

96% alcohol . . . . . .75 cc
Neutral formalin . . . . . .25 cc

The smears in this mixture for fixation in the above solution required fifteen to twenty minutes.

These are the fixation techniques used by us. We have had no experience with the fixation by desiccation described by some authors. The inconveniences of the latter technique will be stated elsewhere.

Lately Brudenell (2) has developed a method, different from those commonly used. He uses Mayer's haematoxylin and eosin for staining. He fixes in Schaudin's solution for ten minutes. Likewise we have had no experience in this technique. Summarizing we may say that all the procedures used and mentioned above are equally good for the staining methods given.

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CLAUDE GOMPEL
Brussels, Belgium

Fixation techniques using alcohol and ether are unchallenged at the present time for routine procedures in gynecological cytology. Some modifications of the usual fixative (equal parts of 95 per cent ethyl alcohol and ether) in which ethyl alcohol is replaced by isopropylic alcohol have been proposed.

This fixative gives very good results because it does not have any chemical action on cells but works only by precipitation and dehydration of cellular components: a further advantage is the transparency of the cytoplasm obtained with high alcoholic stain after this fixation.

Other fixation techniques have been proposed and some are very useful when other staining procedures are used.

We will briefly mention these fixation methods and their applications. For more detail we refer the reader to the original papers.

1. Air drying method.

The smear is allowed to dry in air and may remain dry for approximately 24 hours. The advantages of this method are: the physician is not bothered by problems of fixation and preservation of smears and transportation is easy. Disadvantages are poor fixation of cellular components. The method is not recommended when high alcoholic stains are used (Papanicolaou and Shorr).

The method is indicated when the following staining procedures are used:

a. Iodine vapor stain method for cellular glycogen (1).
b. Pineda's method for cellular glycogen (2).
c. Modified Best's carmine method for cellular glycogen (3).
d. Lison and Vokaer's method for cellular glycogen (4).
e. Silver stain methods for cancer cells (5) (6) (7).
f. May Giemsa method for cancer cells (8).
g. Mayer's method for cervical mucus (9).
h. Various nucleo-cytoplasmic staining procedures using alcohol as solvent which can be used after air dry fixation (10) (11) (12). These methods from our own experience are not as good as the routine Papanicolaou procedure. A reconstruction of dried smears by rehydration has been proposed to reverse the cellular alterations.

2. Ten per cent Formalin.

Smears are fixed for 15 minutes and rinsed in distilled water. This method is used with the following staining procedures:

a. Silver carbonate method for cancer cells (13).
b. Feulgen reaction for desoxyribonucleic acid (DNA) (14).

3. Ten per cent Formalin (99 parts) Glacial acetic acid (1 part).

This is a very good nuclear fixative but gives no cytoplasmic details as with Papanicolaou's procedure.

One per cent glacial acetic acid can be added to alcohol and ether to eliminate red blood cells in hemorrhagic smears (15).

4. Chloroform (3 parts) Ethyl alcohol absolute (6 parts) Acetic acid (1 part).

The Carnoy's fixative is a fast and good fixative. Nuclear and cytoplasmic structures are well preserved. It is used with methyl green-pyronin staining method for DNA and RNA (16).

5. Zenker-Ten per cent Formalin (Helly's method).
   Used in connection with methyl green-pyronin method (16).

6. 95 per cent ethyl alcohol (2 parts) Acetic acid (1 part).
   Used in connection with Black Sudan B. method for cancer cells (17).

7. Picric acid saturated aqueous solution (75 parts) Formalin CP (15 parts) Glacial acetic acid (10 parts) Urea (1 part) (Allen's fluid). Used in connection with methyl green-pyronin method for DNA and RNA (16).

All these methods of fixation which have been summarized are of great value for routine gynecological smears. They are useful when specific cytochemical procedures are utilized for the study of various cytological problems. For this reason, the value of these techniques should be recognized by every cytologist.

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NO DISCUSSIONS RECEIVED:

The above papers by Montalvo and Gompel were not discussed. Members of the Academy who wish to discuss these papers may forward their comments to the Editorial office which will publish these contributions in the chapter "Letters to the Editor" in the next issue. (Editor)

* * *

Symposium C

ANDROGENIC EFFECT ON VAGINAL EPITHELIAL CELLS

THE PHYSIOLOGICAL PRODUCTION OF ANDROGENS IN THE NORMAL WOMAN WHICH MAY INFLUENCE THE PROLIFERATION OF THE VAGINAL EPITHELIUM

JOSE BOTELLA LLUSIA* (by invitation)
Madrid, Spain

(A) Physiological Action of the Androgens in the Female.

It is accepted that production of androgens in the female has been definitely established (1,2,3, 6,14). For many years it was believed that these androgens were something like an ancestral residue or an error of nature. Today, this idea cannot be supported; we know that androgens fulfill a physiological purpose in the female organism, as is proved by the following facts:

1) Androgens are intermediate metabolic products (4,5) in the metabolism of other hormonal steroids.

2) In the normal woman, androgens limit the action of excess estrogens, balancing them and producing what has been called steroid homeostasis (11).

3) A cyclic secretion of androgens has been postulated (10). This secretion would intervene in the regulation of ovarian and uterine cycles.

4) We have demonstrated (4) that during the corpus-luteum phase of the cycle, there is a greater absorption of injected androgens. This supports the idea of a physiological transformation of androgens to progesterone. Androgenesis at the expense of 17-Hydroxyprogesterone is also admitted (5). This is the converse of the previous concept, and the change is probably a reversible one.

5) Finally, the stimulating action of androgens on the female libido is today universally admitted, and sustains too the idea of a physiological effect of androgens in the female.

(B) Androgen Productive Organs in the Female Organism.

1) Adrenals: Different authors (3,8,13,15) have proved experimentally and clinically the formation of androgens in the adrenals of normal women. This secretion seems specially important during pregnancy (6), castration and menopause (9,12) and after injection of Chorionic Gonadotropin (3,13) and/or A.C.T.H. (8).

2) Ovaries: The production of androgens by the normal ovary, has been discussed for a long time. At present, this is generally accepted. Berger (1) in 1923, homogenized the hilial cells of the ovary with the Leydig cells of the Testis, demonstrating later (2) their androgenetic action. In 1937, Hill (7) demonstrated in rodents the virilizing action of transplanted ovaries. Finally, Ponse (14) and Plate (12,13) have demonstrated the origin of the androgens from hyperplastic thecal cells. Both hilial cells and hyperplastic thecal cells can be stimulated in their function by the Chorionic Gonadotropin (14,15).

Summarizing, formation of androgens in the normal female organism takes place at the following organs:

1) Adrenals
2) Ovaries
   a) Hilial cells (or sympaticotropic cells)
   b) Hyperplastic thecal cells

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DISCUSSION:

ROBERT WENNER* (by invitation), Basel, Switzerland:

The question of the physiological production of androgens in the female was discussed accurately in my opinion by Botella-Llusia, in light of present day knowledge. However, he did not discuss the second question, of whether these physiologically produced androgens influence the proliferation of the vaginal epithelium or not. In cases with pathologically increased androgen production, i.e., tumors of the adrenals or ovaries—we definitely observe such an influence. The question of the influence of the physiologically produced androgens cannot be answered so easily, however, because the normal cycle is explained by the normal cyclic follicular hormone and progesterone production. The hormones of the ovaries are absent in the menopause and an atrophy of the vaginal epithelium results in the most cases. This fact proves in my opinion that the physiological production of androgens in the female does not influence the proliferation of the vaginal epithelium, since the androgens of the adrenals are not reduced in the climacteric female, but are sometimes slightly increased.

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CLOSING REMARKS:

JOSE BOTELLA LLUSIA:

Dr. Wenner said that I did not discuss the question that androgens induce proliferation of the vaginal epithelium. I have no definite opinion on this particular phase of androgenic effects and too little personal experience to make a definite statement. I believed that this question could be answered better by other Members of the Academy and particularly by George Wied. However, my subject was not the action of androgens, but the physiological production of androgens which may influence the vaginal epithelium. These endogenous androgens may very well influence the proliferation of the vaginal epithelium, but whether or not they do, I cannot say.

However, Dr. Wenner states that in the menopause the vaginal epithelium is atrophic in most cases. In my opinion, atrophy may occur, but certainly not in all cases. The non-atrophic menopausal cell type is frequently found in smears of menopausal and postmenopausal women. I believe that the presence of this non-atrophic menopausal cell type may prove to be a result of effects of adrenal sex steroids (in part androgens) on the vaginal epithelium.

* * *

IS THERE EVIDENCE THAT ANDROGENS MAY BE METABOLIZED TO SUBSTANCES WHICH MAY HAVE ESTROGENIC EFFECT ON THE VAGINAL EPITHELIUM?

ERNST JÜRGEN PLOTZ* (by invitation)
Chicago, Illinois, U.S.A.

An increase of biologically active estrogens after the administration of testosterone to normal males was first observed in 1937 by Steinach and Kun (1). This finding was confirmed by others (2,3,4), who further showed that the estrogenic activity could be recovered from the phenolic fraction of the crude neutral steroids extracted from the urine. In 1939, Nathanson and Towne (5) extended these observations to castrated women. Using counter-current distribution for the estimation of estrogens, Nathanson et al (6) demonstrated that the increase of "fluorogenic phenols," following the administration of androgenic steroids to two female cancer patients, was at least partly due to an increased excretion of estrone and estriol.

The following explanations of these observations were possible: 1) excess of androgens stimulate the secretion of estrogens, possibly within the adrenal cortex, or 2) androgenic steroids are converted by demethylation and aromatization to estrogens.

Subsequent experiments carried out during the recent years have shown that normal surviving ovarian tissue, obtained from non-pregnant individuals before the menopause, is capable of converting testosterone-C^{14} into radioactive estrogens (7). When human ovarian tissue, displaying marked cortical stromal hyperplasia on histological section (obtained from a post-menopausal woman), was incubated with testosterone-4-C^{14}, radioactive estrone, estradiol, and estriol were isolated by carrier dilution technique (8).

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Experiments carried out in the laboratories of the Chicago Lying-in Hospital and the Argonne Cancer Research Hospital established firmly that testosterone-4-C^{14}, administered to a pregnant patient during the 7th week of gestation, was converted to radioactive estrone (9). The site of this conversion is most likely the placenta, since Meyer (10) has shown that placental tissue slices are capable of converting Δ^4-androstenedione into estrone. Similar results have been obtained in in-vivo experiments by Heard et al (11) who injected testosterone-C^{14} to a pregnant mare and recovered radioactive estrone from the urine.

Evaluating the results of these studies, the conclusion is drawn that testosterone may function as a direct precursor of estrogenic substances in the human female during the various stages of her reproductive life.

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DISCUSSION:

JOHN A. FINKBEINER* (by invitation), New York, New York, U.S.A.:

It has been documented chemically, clinically and cytologically that androgens can be converted into estrogens. West et al have reported the conversion of testosterone to estrogens in castrated, adrenalectomized females maintained on 75 mg of cortisone acetate daily. Urinary estrogens were absent during the control period but following the administration of testosterone proprionate, estrone and estradiol were identified by a counter-current distribution paper chromatography technique (1). This is the critical experiment, for studies in patients or animals with intact adrenal or gonadal function, a possible adrenal source of estrogen cannot be ruled out.

Myers et al reported four patients with androgen-induced exacerbation of breast cancer measured by the calcium excretion technique and believed that the conversion of androgen to estrogen was the possible underlying mechanism (2). Vaginal smear data, incompletely reported in that paper, suggest that androgens can be converted to estrogens. This is manifested by a shift in the vaginal smear pattern from a baseline of relatively little activity to one of greater activity, i.e., greater differentiation. See also my discussion of Boschann's paper on "Effect of Administered Androgens in Patients with Atrophic Menopausal Cell Type."

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KARL JUNKMANN* (by invitation), Berlin, Germany:

Plotz discusses a very interesting viewpoint in his above presentation.

During the past few years we have learned from our biochemical studies on human and animal metabolism that cholesterol is definitely metabolized via pregnenolone and progesterone to the C_{21}-steroids. From these C_{21}-steroids, there is another metabolic path to the C_{19}-and C_{18}-steroids.

On the other hand, there is a direct path of synthesis, at least for the adrenal steroids, in which the acyl fatty acids may be synthesized directly to the C_{21}-steroids. However, I feel that this path has not yet been definitely proven, since the present experiments cannot exclude the possibility that the synthetic way leads via cholesterol and pregnenolone, respectively.

Hence, the physiological sequence: "Progesterone and Androgen to Estrogen" is based on the one hand, on the chemical genesis of the hormones. On the other hand, it is based on the fact that ICSH and HCG or PMSG induce an increase of the ovarian androgen production on the hypopysectomized rat in the absence of F.S.H. This seems to indicate that the increased production of androgens is necessary for the subsequent synthesis of estrogens in the ovaries (as well as in the testes).

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J. C. de LAGUNA, Mexico, D. F., Mexico:

In a study that we are doing concerning hormonal action on precancerous lesions, we have observed a clear-cut cytological picture of estrogen effect after the administration of androgens. (E.V.H., age 38) with more than one year of surgical castration and a picture of epithelial atrophy underwent treatment with 25 mg of testosterone propionate per week, for 6 weeks. Starting at the third week of testosterone administration, the cytological smears consistently showed 100 per cent superficial cells, with a predominance of precornified cells similar to a late regressive stage. On discontinuing the treatment, the picture of atrophy reappeared.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

We can accept as proven, the concept that testosterone as well as other hormones can be transformed in the female to compounds with opposite hormonal action or can stimulate the production of antagonistic hormones in other organs. But I think that this phenomenon in general has no practical importance in hormonal treatment of the female. The possibility is suggested by the chemical similarity of the sex hormones and by the general laws of sex endocrinology. I think, however, that we should examine the conclusions of some workers. First, the conclusions of Nathanson and Towne concerning the estrogenic reactions of the vagina after testosterone administration were never confirmed by other authors and seem to result from a defective cytological technique (3,4). Concerning the experiments with radioactive C 14: may we completely exclude the possibility that the radioactivity which normally does not exist in hormones may act as a catalyst, for the conversion of these hormones to other compounds? Personally, I would never perform experiments on pregnant women with radioactive substances. On the other hand, we must ask for experiments with non-radioactive hormones, which are highly purified. I remember the important battles which Courrier had to fight 20 years ago in the problem of the hypothetical progesterone-like activity of testosterone. As for testosterone I have found that the cytological reactions in the vagina can show more or less different reactions with the same propionate, coming from different pharmaceutical laboratories. If ordinary testosterone in the woman gives no clear cytological demonstration of partial estrogenic activity perhaps because the degree of conversion is insufficient, I would remember the important observations of Ghilain and Peers (1) which I confirmed in my own experiments: viz., in the normal pregnant woman, diethylstilbestrol orally produces no estrogenic reaction in the vagina. If an estrogenic reaction occurs, it heralds an impending miscarriage. Estrogens produce, if the ovum is still alive, progesterone-like reactions, whereas the same estrogens administered locally always produce estrogenic reactions, as in the non-pregnant woman.

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A. E. RAKOFF, Philadelphia, Pennsylvania, U.S.A.:

There can be little doubt from the isotope studies quoted that androgens are in part converted to substances having estrogenic activity. In most studies the proportion of resulting estrogenic activity is relatively small. In the human castrate the estrogen level is usually very low but may increase, presumably as a result of secondary adrenal cortical hyperfunction. Present evidence would suggest that in the latter circumstance the increased estrogen level does not result from the conversion of androgens but rather increased estrogen excretion by the adrenal cortex. The amount of androgen secreted by the normal female is not usually of an amount sufficient to significantly alter the vaginal smear pattern.

JOSE R. del SOL* (by invitation), Madrid, Spain:

The very interesting and important findings of Plotz bring up some questions. We know from our cytological studies that a distinct percentage of patients with an atrophic menopausal cell type will not respond with a proliferation of the vaginal epithelium upon administration of androgens, whereas the majority of the cases will show an intermediate type of induced cell growth. I would like to ask Plotz if he might offer an explanation for the fact that some patients with an atrophic menopausal cell type do not show any proliferative change after androgen administration. If androgens were metabolized to estrogens, would it then not be logical that we would see in every case a distinct type of induced proliferation since we know that the atrophic epithelium will always respond to estrogens?

The other question which is raised is this: after androgen administration in a patient with an atrophic menopausal cell type one rarely observes a cytological proliferation which is definitely estrogenic, i.e., acidophilic cytoplasm of flat singly lying cells with pyknotic nuclei. I would like to ask Plotz if he believes that this is due to the fact that only a part of the androgens will be metabolized to estrogens whereas the non-metabolized portions of the androgens would suppress the occurrence of an estrogenic cell type?

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ROBERT WENNER* (by invitation), Basel, Switzerland:

The author discusses extensively the problem of the metabolism of androgens to estrogens in the female organism and refers to the related literature. However, he does not discuss the question at hand which deals with the effect of these metabolized androgens, on the vaginal epithelium.

Rubinstein and co-workers(6) found in the year 1938 that testosterone propionate induces estrus and aperture of the vagina in the infantile animal. It was shown previously in the year 1937 by McEven and co-workers (3) that the induced effect (i.e., epithelial cornification) in the infantile animal may be obtained only on animals which have not been castrated. In controversy with the latter findings, it was shown by Korenchevsky and co-workers (2) that an existing estrus may be suppressed by androgens.

Berthier and co-workers (1) as well as I could show (7) that the occurrence of epithelial atrophy in castrated animals or menopausal women may be inhibited by administration of androgens. We showed in a series of extensive cytological and histological experiments on rats that androgens induce a proliferative effect similar to that induced by follicular hormone. At this time, we were assured that these effects were a direct result of androgen administration. Pundel (5) describes in his new monograph the characteristic appearance of the androgenic smear type.

If one attempts to synthesize the above experimental facts and the results of the experiments by Plotz (4) then we would arrive at the possibility not yet fully established that proliferative changes on the vaginal epithelium after androgen administration are induced as a result of metabolism of these substances to estrogens.

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CLOSING REMARKS:

ERNST JÜRGEN PLOTZ:

The interesting comments and stimulating questions raised by the discussants give me a good opportunity to re-evaluate the results of some observations pertinent to our problem. I fully agree with Junkmann, that besides the sequence cholesterol, pregnenolone, progesterone, 17-hydroxyprogesterone, androstenedione-estrone, other pathways of estrogen synthesis may be available in the human organism.

It seems to be generally accepted that such androgenic hormones as testosterone and androstenekione can be converted into estrogenic hormone, for instance estrone, estradiol and others, in the human female organism. The results of isotopic studies carried out with radiochemically pure compound in vivo and in vitro demonstrate this conversion almost beyond doubt. To my knowledge, there is no evidence whatsoever, that radioactive carbon "may act as a catalyst" in such a conversion, as suggested by Pundel.

The sites of androgen-estrogen conversion are the pre- and post-menopausal ovaries, the placenta and—in all likelihood—the adrenal cortex. Finkbeiner mentions the interesting studies of West et al. I think their results are strongly suggestive for an androgen-estrogen conversion in bilaterally ovariectomized and adrenalectomized women with metastatic breast cancer, but they raise the question whether other organs—besides endocrine glands—possess enzyme systems capable of converting androgenic into estrogenic hormones. On the other hand, it is possible that accessory glandular tissue was left after the operations. If this were true, we would be unable to decide whether the administered testosterone stimulated the production of estrogens by this rest tissue or was converted into estrogens within this tissue. The latter possibility is the most likely one.

As Rakoff pointed out in this discussion, the yield of estrogens derived from androgens is reportedly relatively small in most studies, usually less than 1%. In our own study of a pregnant patient (scheduled for therapeutic abortion), we recovered only 0.8% of the administered radioactivity in the phenolic fraction. The low yield of estrogenic hormones may explain the fact that one seldom observes the typical estrogenic response of the vaginal epithelium after the administration of androgenic hormones. Del Sol raises the interesting question about whether the absence of a typical estrogenic proliferation of the vaginal epithelium after the administration of androgens to patients with an atrophic menopausal cell type is due to the fact that only a part of the androgens will be metabolized to estrogens whereas the non-metabolized portions of the androgens would suppress the occurrence of an estrogenic cell type. I am certainly not in the position to give a competent answer to this question, but I feel inclined to say the so-called androgenic proliferation may be due to the combined action of non-metabolized androgens and newly formed estrogens on the cell metabolism. Basic investigations of the mode of action of steroid hormones on the molecular level in body cells may help to elucidate this problem.

It seems logical to assume that any definite increase in the percentage conversion of androgens to estrogens would favor the appearance of an estrogenic cell type in the vaginal epithelium, a possibility which is supported by the observation of de Laguna and others. For the same reason, I am somewhat re-

luctant to accept Pundel's verdict that the conclusions of Nathanson and Towne concerning estrogenic reactions of the vagina after testosterone administration seem to be the result of a defective cytologic technique. Variations in the percentage conversion of androgens to estrogens should be expected under various physiologic and pathologic (cancer!) conditions. A complete lack of androgen-estrogen conversion due to defective enzyme systems in aging endocrine glands can be offered as an explanation for the observation of del Sol that some patients with an atrophic menopausal cell type do not show any proliferative changes after androgen administration.

* * *

THE EFFECT OF PHYSIOLOGICAL SEX HORMONES ON THE VAGINAL EPITHELIUM OF PATIENTS WITH INACTIVE OVARIES

GEORGE L. WIED
Chicago, Illinois, U.S.A.

Which cytological smear type persists in patients with inactive ovaries? This question may be answered by considering the patient in whom both ovaries have been surgically removed. If it were true that ovarian hormones were the only hormones responsible for the proliferation observed in the vaginal epithelium, then one would expect to find an atrophic smear type in every surgical castrate. This is, however, not so. In point of fact, one will find an intermediate type of proliferation, consisting of intermediate or superficial non-cornified cells with moderate folding of cells and rather little crowding of cells (Fig. 1) (6), in the majority of patients who have been surgically castrated during their reproductive years.

I believe (8) that there exist only two absolutely diagnostic "hormonal smear types": 1) the highly proliferated cell type (consisting predominantly of superficial cornified cells with pyknotic nuclei, acidophilic cytoplasm, flat and singly lying cells) which definitely indicates estrogenic activity, and 2) the atrophic cell type (consisting predominantly of parabasal atrophic cells) which definitely indicates lack of estrogenic effect on the vaginal epithelium.

The intermediate stages of proliferation between these above cell types are per se essentially non-specific (8). However, these intermediate stages of proliferation may permit some relatively diagnostic conclusions if the age of the patient, the menstrual history, previous hormonal therapy, previous surgery and individual variations in response are considered by the cytologist.

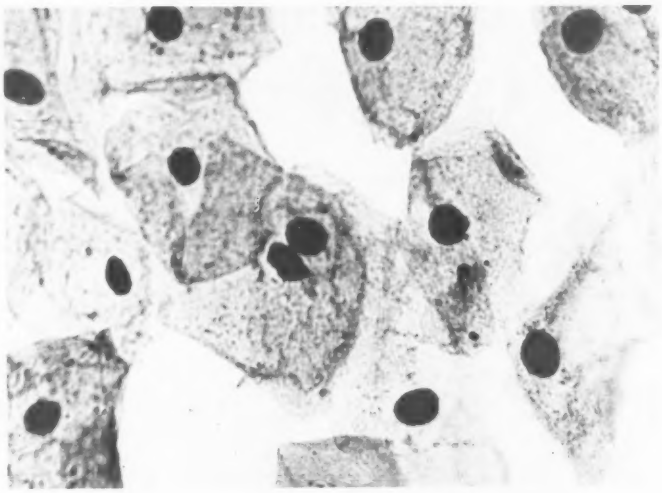

Fig. 1. Vaginal smear of surgical castrate 18 years after surgery. The smear exhibits mostly superficial non-cornified and intermediate cells, no leukocytes and healthy vaginal flora. This physiological proliferation seems to be due to steroid hormone stimulation from the adrenals.

I do not believe that the vaginal epithelium has a "proliferative potency" (1) of its own since the relation of atrophy to proliferation changes in castrates if one also removes the adrenal glands and/or the pituitary gland (2). If the vaginal epithelium possessed such a proliferative potency, independent of sex hormones, then one would expect this quality to manifest itself after adrenalectomy and/or hypophysectomy in these castrated women.

One is rather safe in concluding that the adrenal hormones are responsible for the proliferation observed in the vaginal epithelium after the surgical removal of the ovaries. This does not deny that the adrenals participate in the proliferation of the epithelium during the unreproductive period when one or both ovaries are functioning. After the removal of the ovaries, however, the adrenals are the only glands ("third gonad" of Botella) left to produce sex steroids in appreciable amounts and to induce proliferation of the vaginal epithelium. Androgens comprise the majority of sex steroids produced by the adrenals.

Can it be assumed then that intermediate proliferation found in the majority of surgical castrates (attributed to the action of adrenal sex steroids) is an androgenic type of proliferation?

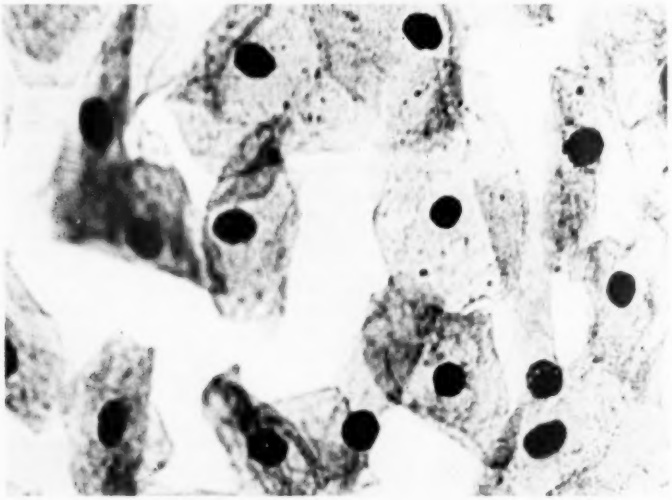

Fig. 2. Vaginal smear of a surgical castrate who exhibited prior to androgen administration the atrophic menopausal cell type. This smear shows the induced proliferative changes after injection of 100 mg testosterone propionate. The induced type of proliferation is similar to the above described (Fig. 1) physiological type of proliferation.

This question may be partly answered by the consideration of androgen therapy in senile surgical castrates with an atrophic menopausal cell type. Following the injection of androgens into these patients, the atrophic smear type is replaced in the great majority of cases by an intermediate type of proliferation consisting of intermediate or superficial non-cornified cells exhibiting slight crowding and moderate folding (Fig. 2) (3,4,5,7,8). This is practically the identical cell type observed in most of our patients surgically castrated during their reproductive years.

The similarity of these findings: 1) after surgical removal of the ovaries, in previously normally menstruating women, and 2) after androgen administration in menopausal patients and castrates with atrophic menopausal cell types, seems to substantiate as far as is cytologically possible that the smear type observed after removal of the ovaries is predominantly "androgenic proliferative type."

The following problems warrant further consideration: 1) Why does not androgen administration induce proliferative changes in some patients with an atrophic smear type? 2) Why do some few menopausal patients, with an initial non-atrophic menopausal smear type, show epithelial regression to complete atrophy with long-term androgen therapy?

Summarizing, I would point out that insofar as valid cytological observations can be made at all as to what constitutes a purely or predominantly estrogenic or progestational effect, so also can these observations be made for androgenic effects. By excluding known possible hormonal influences, we can conclude that the cytological smear type in a surgical castrate is induced by sex steroids of the adrenals, apparently by predominantly androgenic substances.

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PETER STOLL
Heidelberg, Germany

We have assessed the endocrine function in menopausal women by means of cytology. These cytological studies were performed on 882 patients during the years 1952-1956. In all cases, at least two years had elapsed since the onset of the menopause. Three smears were taken from each patient at four day intervals. The results are as follows:

	No. of patients	Estrogenic	Androgenic	Atrophic	Undetermined
Normal post-menopausal	516	94 18.2%	258 50%	141 27%	23
Benign Tumors (Myoma polyps)	168	40 24%	91 54%	37 22%	-
Carcinoma of corpus uteri	98	18 18%	45 45%	30 30%	5
Carcinoma of cervix or vagina	100	25 25%	43 43%	30 30%	2

No significant deviation was found in any of the above individual classifications when the different groups of patients were compared.

JEAN BERGER
Basel, Switzerland

If we want to examine cytologically or otherwise the influence of sexual hormones on patients with so-called "non-active ovaries," we have to state first a few conditions.

We should be aware that ovarian function almost surely never stops, not even after the menopause (Botella-Llusia, Pundel, Zinser and others). Therefore, the effect of sexual hormones should be evaluated in the vaginal smear of the following groups only:

a) in old women, who are at least 15 years menopausal. They should be tested prior to the experiment by vaginal smear and 24-hour urinary hormonal output.

b) in women castrated by operation. In these patients the pre-test level also has to be recorded, since the so-called "sexual zone" of the adrenals is able to produce sexual hormones (Botella-Llusia, Reichstein, Beall, Pfiffner).

The influence of the single hormones is again dependent on the concentration, dose, and application of the drug. Like Zinser, we too, obtained in women long in the menopause, with initial atrophic smears, the following results:

1. After injection of estrogens: Some days after application, the smear appeared "cleaner" and a reduction of leukocytes was noted. After further application, an increase in cells of the intermediate layer with some superficial cells occurred following which an increased appearance of acidophilic superficial cells with pyknotic nuclei was observed. Concomitantly, the hormonal analysis showed in the 24-hour urine a marked increase of the phenol-steroids.

2. After injection of progesterone: A few days after the application of 20-40 mg a definite increase of intermediate cells with crowding, is seen. The leukocytes diminish gradually. Finally, we find a mixed picture with intermediate isolated basal and acidophilic superficial cells.

3. After injection of androgen: The basal cells become more marked after a few days application of 5-20 mg of perandren (CIBA). After further application intermediate cells appear. Rarely are superficial cells observed. The results of our own examinations concerning the application of ACTH and LH to surgical castrates may be of some interest. In 6 cases of surgically castrated women three times fifteen LU ACTH were injected. Two days later we noted a definite increase, not only of the 17-ketosteroids, but of the urinary output of estrogen too. In spite of the output of estrogen, only a small effect could be noted in the vaginal smear. More intermediate cells appeared however, and the picture was similar to an "androgen smear" (corresponding to the increased output of the 17-ketosteroids).

After administration of high doses of pregnyl (LH) neither biological testing not the vaginal smear showed evidence of estrogen effect. The smear remained atrophic.

We believe that at this time no final conclusions on the effect of sexual hormones in women with inactive ovaries are warranted. This problem has to be studied by further experiments, especially in castrated women. (See figures 1-4.)

DISCUSSION:

H. WERNER BOSCHANN* (by invitation), Berlin, Germany:

One might also consider the question of how much a subthreshold estrogenic effect may be responsible for the intermediate type of proliferation. The possibility of such an effect may be supported by

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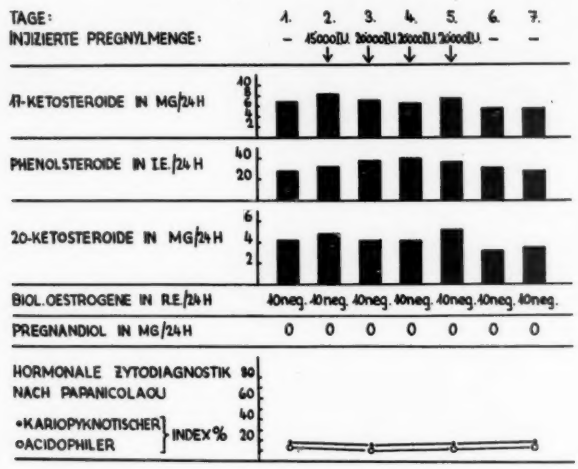

Fig. 1. Charge with LH of castrated woman, 21 years old. (Jean Berger)

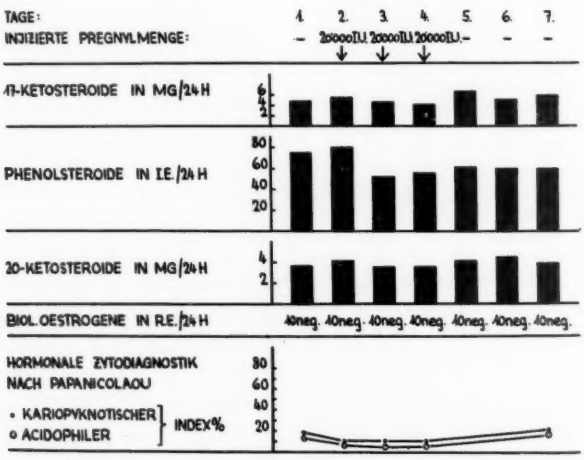

Fig. 2. Charge with LH of castrated woman, 51 years old. (Jean Berger)

the experience that one finds very similar cellular patterns in postclimacteric women with "androgenic proliferation" and in patients with secondary amenorrhea and some forms of primary amenorrhea (with breast development indicating estrogen production). One might explain the fact that one does not find a definite estrogenic cell type in those cases because there is a local antagonism of androgens and estrogens in the vaginal epithelium and that possibly the unmetabolized androgens are antagonistic to the relatively small portion of androgens metabolized to estrogens, and, therefore, inhibit the occurrence of estrogen induced cellular changes. Following this train of thought, it would not be too astonishing to find some superficial cells with pyknotic nuclei after test administration of androgens in patients with an initially atrophic cell type. If one administers mixtures of estrogens and androgens one will find that the karyopyknotic index increases with the relative amount of administered estrogens. I believe that the proliferated cell types in patients with inactive ovaries are due with a high degree of probability to androgens. However, it is possible that in addition to that the proliferation may be stimulated by metabolites of the androgens which might be estrogenic. The latter portion will be considerably smaller and may be negligible.

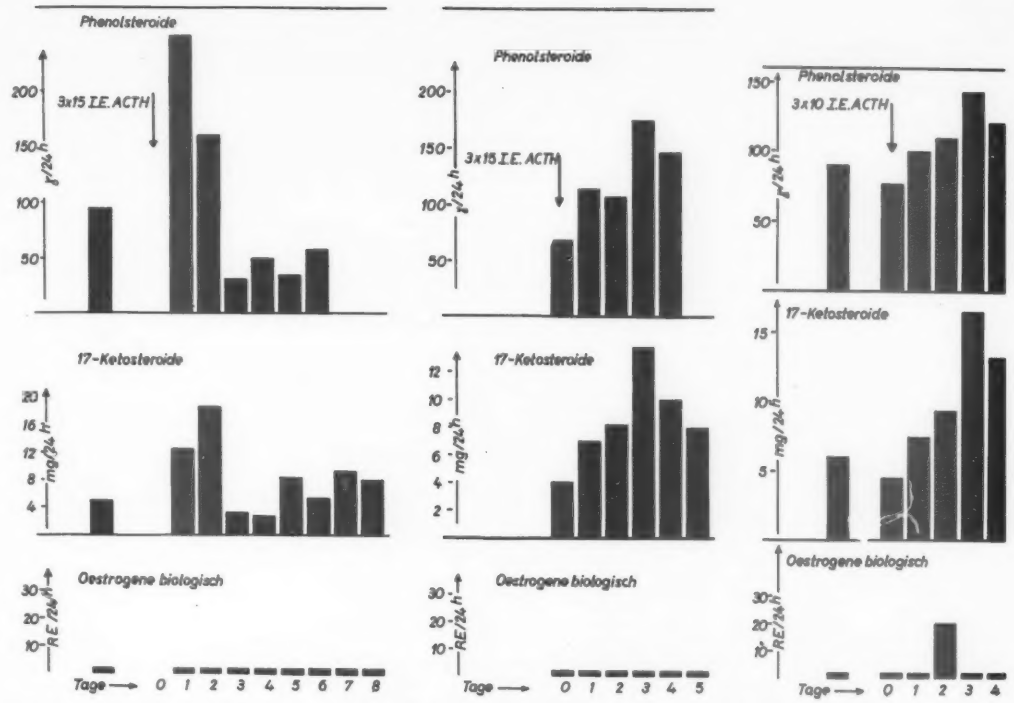

Fig. 3. Charge with ACTH of three castrated women. Patient in left column is 32 years old, the patient in middle column is 48 years old and the patient in the right column is 50 years old. (Jean Berger)

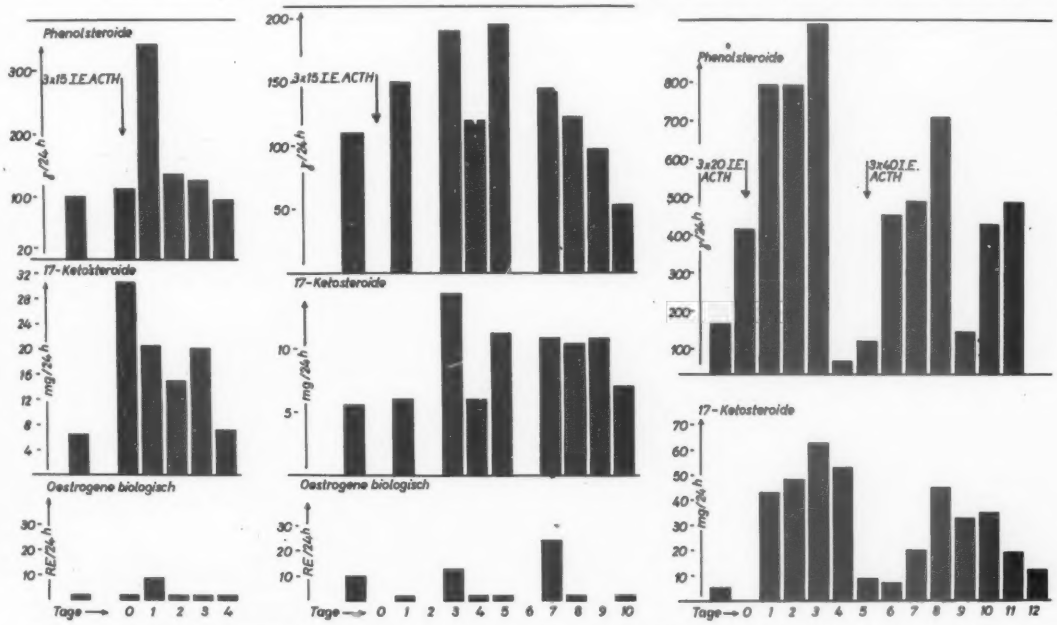

Fig. 4. Charge with ACTH of three castrated women. The patient in the left column is 24 years old, the patient in the middle column is 36 years old and the patient in the right column is 31 years old. The smears of the six castrates (Figs. 3&4) exhibited the androgenic cell type with many parabasal and intermediate cells in evidence. (Jean Berger)

JACQUES FERIN* (by invitation), Louvain, Belgium:

Among 70 cases of bilateral oophorectomy, we have seen 32 times an atrophic smear type, 21 times an intermediate type of proliferation, and 17 times an estrogenic type of proliferation with more than 5% karyopyknotic cells (1).

The chemical determination of urinary estrogens, with the most refined technics (2) (3), in the surgical castrate often gives positive results.

These estrogenic substances may arise from little ovarian remnants, from adrenals, directly or after conversion of androgens to estrogens, or possibly even from the digestive tract [urinary estrogens after bilateral oophorectomy, bilateral adrenalectomy and hypophysectomy (3)].

We must not forget that the atrophic vaginal epithelium reacts to very small estrogenic doses: 10 mcg. estriol/24 h. (4), and this may persist for a week after treatment.

For these reasons, we feel that the intermediate proliferation seen in the surgical castrate is a mixed type of proliferation: androgenic + estrogenic.

Similar smears are regularly obtained in the surgical castrate by various doses of androgens and estrogens simultaneously administered (5).

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JOHN A. FINKBEINER* (by invitation), New York, New York, U.S.A.:

Based on the experience gained from examination of serial vaginal smears obtained from several hundred women with advanced breast cancer who have been subjected to oophorectomy, adrenalectomy, or hypophysectomy, I cannot agree with Wied's answer in several regards.

In the first place, there has been no agreement on what constitutes "inactive ovaries." Is it the woman who incurs a natural menopause? Certainly we all have seen vaginal smears of women twenty or thirty years or longer after the last menstrual period, who exhibit a rather striking degree of estrogenic activity. Or is it the patient who has a completely atrophic smear? Is the response to estrogen, to progesterone, and to androgen the same in these two groups of patients—certainly not! The magnitude of response certainly varies considerably in various groups of patients presenting identical baseline smears and receiving uniform amounts of the identical hormone preparations by the same route and duration of administration.

Much more complex is the response to oophorectomy, adrenalectomy, and hypophysectomy. Serial pre and post operative smears in over four hundred women undergoing oophorectomy for treatment of mammary cancer, show a wide atrophic smear within a few weeks or months following oophorectomy. Other women, a few months after oophorectomy have a level of vaginal smear activity equivalent to that of a normally menstruating woman at the height of her cycle. At adrenalectomy, there was marked adrenal cortical hyperplasia in such patients. One woman even developed, quite acutely, a classical and fulminating Cushing's syndrome. Space does not permit discussion of the wide range of response observed in over 200 patients undergoing bilateral adrenalectomy and over 100 women undergoing hypophysectomy. Suffice it to say that the results are much more complex and difficult to interpret, especially since both of these procedures necessitate replacement hormone therapy.

But can the response to exogenous hormones in these patients be compared to the response to a normal individual or to one with intact but "inactive" ovaries? And if by chance, the vaginal smear pattern is similar in two physiologically dissimilar situations, does it mean that the smears are identical? As I have stated elsewhere in this symposium, it is too much to expect that vaginal epithelium to be a quantitative and qualitative bioanalyzer.

The two additional problems that Wied has observed are confirmed and these observations emphasize the point that I have made elsewhere in this symposium: one cannot assume that the proliferation of the vaginal mucosa that usually occurs in patients receiving androgen is due specifically to the androgen and not due to its metabolites. Rather, these two observations may be the true androgenic effect, i.e., an actual anti-estrogenic action, and the more common vaginal smear response may be due to metabolic transformation of androgen to a low estrogen level.

I see no reason to call this non-specific proliferative type of smear an "androgenic type" and would reserve that term for a specific cytological pattern that can be seen in patients receiving androgens or in patients with masculinizing adrenal or ovarian tumors.

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E. JÜRGEN PLOTZ * (by invitation), Chicago, Illinois, U.S.A.:

As Wied has pointed out, there seems to be no doubt that, even after the removal of the ovaries, the vaginal mucosa remains under the influence of certain steroid hormones, the source of which is most likely the adrenal cortex. The nature of these steroid hormones is difficult to determine, since the response of the vagina to these compounds, as evaluated by cytological criteria, is limited to certain morphological changes, which may lack specificity in regard to the various types of steroid hormones. The adrenal glands are capable of producing a variety of hormones: 1) cortisol and related steroids which have a catabolic effect on protein metabolism and promote gluconeogenesis; 2) aldosterone, which has a powerful effect on electrolyte and water metabolism; 3) androgens, which besides their specific action on secondary sex organs have a pronounced anabolic effect on protein metabolism; and 4) estrogens and progesterone, the latter being an important intermediate in the biosynthesis of cortisol and related hormones. Furthermore, it is entirely possible that some of these hormones, for instance, androgens, can be partially converted to estrogenic hormones, which then contribute to a proliferative effect on the vaginal mucosa. Thus, the extent to which the vaginal cell layers (impedients with inactive ovaries) are influenced by adrenal steroids depends on the following factors: 1) rate of secretion of the different compounds by the glands, 2) prevalence of one or the other hormone, 3) interconversion of hormones, and 4) changes in the responsiveness of the vaginal cells to such hormonal stimuli. Basic investigation of the mode of action of steroid hormones on the molecular level in the cells is necessary to elucidate these problems.

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GUILLERMO TERZANO, Buenos Aires, Argentina:

After a menopause valuable pictures have been described in vaginal smears by Papanicolaou and Shorr (1936), Fontana (1940), Papanicolaou and Traut (1943), Ferin (1945), Kernodle and Cuyler (1948), Bourg and Pundel (1950), Allende and Orias (1950), etc.

As long as 30 years after the menopause, we have found atrophic vaginal smears in 77.8% of the cases, and smears showing some evidence of proliferation in 32.2%. The smears were reported according to the degree of proliferation of the epithelium.

The idea of Wied sounds excellent. It could explain in satisfactory fashion why we find those non-atrophic vaginal smears so many years after the menopause.

(The paper by Berger was not discussed. Members of the Academy who wish to discuss this particular paper may send their discussions to the editorial office which will publish these comments in the next issue under "Letters to the Editor"—Ed.)

CLOSING REMARKS:

GEORGE L. WIED:

The subjects on which we do not agree entirely are the most fascinating because they show where further research could be applied to clarify our knowledge and to obtain uniformity in terminology.

Nowhere can I disagree with Boschann's discussion. It is entirely possible that estrogens participate partly or mainly in the proliferation of the epithelium in the patient with secondary or even primary amenorrhea who are apparently under estrogen stimulation, and who exhibit a smear type practically identical to that observed in post-climacteric years. I would go even further than Boschann, and say that some of these amenorrhoeal patients exhibiting this particular type of intermediate proliferation may have a rather high estrogenic level. They may not show high proliferation to the cornified cell layers because the estrogenic effect has been active for a long period of time in the same high or similar high dosage. The "long-term effect" of a constant estrogen dosage usually results in gradual regression in the height of proliferation to the non-cornified superficial and intermediate cell layers and bacterial cytolysis may be observed. In an examination of 120 patients who were longer than one year after hysterectomy and oophorectomy under permanent estrogen therapy 1 mg. diethylstilbestrol per os daily, 87 showed an intermediate type of proliferation without karyopyknosis, 23 showed various degrees of karyopyknosis, and only ten showed the typical estrogenic pattern with flat, singly lying cells, acidophilia and a considerable degree of karyopyknosis. "Short-term" administration of 1 mg. diethylstilbestrol daily on the other hand induces in over 80% this latter pattern. In other words, an adequate dosage of estrogens for a short period of time would result in cornification of a previously atrophic smear type whereas the same dosage administered over a long time will result in an intermediate type of proliferation in the vast majority of the cases. This indicates that the "end organ" is developing a "tolerance" to a constant dosage and that a larger dose of estrogen might be necessary to maintain cornification. In primary amenorrhea with estrogen production the patient may therefore produce considerable dosages of endogenous estrogens which apparently exist over a long period of time (as evidenced by the mentioned breast development). In the case of exogenous long-term estrogen effect one can increase the dosage to maintain high proliferation. The above amenorrhoeal patient cannot increase her endogenous estrogen production to guarantee maintenance of the high degree of proliferation. I offer this as a possible explanation of the findings of Boschann which are in my opinion entirely correct.

The figures of Ferin correspond by and large with our figures. I am sure that Dr. Ferin has based his classification of smear types as "estrogenic" not merely on the fact that the smears exhibited a karyopyknotic index of over 5%. One may observe such indices sometimes after local androgen administration which seem to be, therefore, a "true" androgenic effect. In a series of 50 tests of local androgen

administration (testosterone) we found that the karyopyknotic index was elevated over 5% in 9 out of 50 cases. Within the range of individual variation this relative figure compares favourably with the figures observed by Ferin on surgical castrates without androgen administration (17 out of 70 showed karyopyknosis over 5%). I concede that Ferin's opinion that the intermediate type of proliferation may be a result of androgenic and estrogenic effects may be correct. As a matter of fact I would even say that it is highly improbable that it would be a purely androgenic effect. I believe, however, that the physiological proliferative type in castrates is predominantly induced by adrenal androgens in varying degrees.

Finkbeiner is correct when he says my statements are the result of examinations on surgical castrates who do not necessarily represent "women with inactive ovaries." I reported here on surgical castrates intentionally as I do not know when intact ovaries are "inactive." My purpose was to demonstrate that the ovaries are not the only glands which induce proliferation. I believe that I have obtained "clean" experimental conditions by using patients in whom the ovaries have been removed. The criticism by Finkbeiner is, however, astonishing because he apparently bases his present statements on physiological hormonal stimulation of the vaginal epithelium on experiences with patients with advanced breast carcinoma who are unphysiological to say the least. Finkbeiner's experiences with patients with advanced breast carcinoma may be extensive, but they have another "unknown" and represent certainly within this subject "unclean" experimental conditions. Moreover, Finkbeiner seems to disagree with himself. Let me repeat some of his own statements: he speaks of cytological evidence "of rather striking degree of estrogenic activity" in one sentence, and says in another sentence that "it is too much to expect that vaginal epithelium be a quantitative and qualitative bioanalyzer." Finkbeiner's suggested terminology at the end of his comment does not agree with the tenor of his discussion, nor—I believe—will it agree with the overwhelming majority of the endocrinological cytologists. Finkbeiner wants to "reserve that term (of "androgenic cell type") for a specific cytological pattern that can be seen in patients receiving androgens or in patients with masculinizing adrenal or ovarian tumors." Much could be said in answer to this suggestion: there is no such thing as a specific cytological pattern which occurs always after androgen administration. For instance, after treatment with androgens some patients with atrophy remain atrophic while the majority shows proliferation, some to the superficial basophilic cells, some even with pyknotic nuclei. The same holds true for patients with masculinizing tumors: there is a wide range of cytological response, from atrophy to rather high epithelial proliferation. If one were to adopt this terminology suggested by Finkbeiner which seems based on clinical facts, but certainly not on cytological features, then one has a mix-up of clinical information with non-specific cytological findings.

Plotz in his very worthwhile discussion makes it clear that we have just scratched the surface in some endocrinological and cytohormonal problems.

Our figures on the relation of atrophy to proliferative stages in post-menopausal patients are not entirely identical to those of Terzano. Our figures more closely agree with Stoll's figures. Atrophic smears are found in the minority in our material as compared with the proliferative stages. There are many questions open, and much research can be devoted particularly to the problem of physiological hormonal stimulation of the vaginal epithelium.

* * *

IS THERE A PHYSIOLOGICAL CELL TYPE WHICH MAY BE DEFINED AS "ANDROGENIC CELL TYPE"?

J. PAUL PUNDEL
Luxembourg, Luxembourg

In order to answer this question one must first consider experimental studies comparing the typical actions of the different sexual hormones in the castrated woman showing a minimum of endogenous hormonal production. In general, we have to consider only estrogens, progesterone, and androgens such as testosterone or androsterone.

The vaginal epithelium has the following properties: proliferation, differentiation and desquamation. The differentiation begins in the upper part of the basal layer whose essential activity is proliferation. It can show the formation of an intermediate layer with marked glycogenization and then the formation of a superficial layer characterized by the appearance of karyopyknosis and eosinophilia of the cytoplasm, with a simultaneous diminution of the glycogen content. The desquamation can present various degrees and types: shedding of isolated cells or clusters and the desquamated cells can present a more or less marked folding of the cytoplasm. We know now that practically all sexual hormones can produce with sufficient doses a very similar proliferation in the basal layer, and the differentiation of a more or less appreciable intermediate layer with marked glycogen deposition. The degree of proliferation and also the amount of glycogen in the vaginal cells or epithelium can consequently not be accepted as a criterion for the particular activity of a single hormone. So it is absolutely incorrect to correlate the degree of proliferation with a graduation of estrogenic activity.

Estrogens furthermore produce the development of a superficial layer with karyopyknosis and eosinophilia. We were never able to produce experimentally the appearance of eosinophilic and/or karyopyknotic superficial cells (nucleus less than 6mμ in diameter) with hormones other than estrogens, and we can consider these modifications as a specific criterion of estrogen activity. Progesterone in massive doses can produce the beginning of a superficial layer, with a beginning of karyopyknosis, but in general without eosinophilia. The most important vaginal effect of progesterone consists in the accentuation of the desquamation in thick clusters and folding of the cells, while under the estrogen and androgen effect the cells desquamate in

general in single and more flat elements. Progesterone has also a synergistic effect on estrogens (and also to androgens) in accentuating the proliferation and the formation of a thick intermediate layer. Its antagonistic effect on estrogens consists of promoting mass exfoliation resulting in an apparent regression due to massive shedding of the superficial layer.

Testosterone produces a marked proliferation of the basal layer and the formation of a thick intermediate layer with marked glycogenization. It never produces karyopyknosis and eosinophilia in the superficial layer while acting alone. Its desquamating effect is less than that of estrogens and progesterone. The characteristic effect of testosterone, the typical androgen, results in proliferation without differentiation to cornification and karyopyknosis. The vaginal cells under pure androgenic stimulation are exclusively of the parabasal or intermediate type, with the high glycogen content in the cytoplasm producing a pale basophilic staining of the cytoplasm. The nucleus remains large and stains pale with chromatin disposed in very minute granules. Determination of the D.N.A. content of these nuclei under microphotometry seems to indicate a relative lower D.N.A. content compared with that of nuclei from the same cell type under pure estrogenic stimulation.

As estrogens produce a progressive diminution of the nuclear diameter and a progressive condensation of the chromatin, resulting finally in complete pyknosis, beginning at the top of the intermediate layer, it is possible to distinguish between androgen-influenced and other cells effected by estrogen and progesterone. (Progesterone produces a similar progressive modification of the nucleus, but without complete pyknosis.) In general, however, it is not possible to distinguish between progesterone and estrogen influenced intermediate cells, since nuclear differences at this level are minimal. The cytological characteristics of the androgenic cells appear vividly after staining with the Shorr S30hematoxyline technique, whereas the Papanicolaou technique occasionally shows a light eosinophilic reaction of the cytoplasm and a more compact nucleus. To date, we are able to produce this androgenic cell type only with pure testosterone or the testosterone propionate or testosterone acetate while other hormones with androgenic activity frequently produce a more or less multihormonal effect.

The combined administration of testosterone and estrogens shows that a synergism exists in the proliferative effect of these hormones, but an antagonistic effect is present in the differentiation, since testosterone can neutralize the estrogenic differentiation in superficial eosinophilic and karyopyknotic cells. If the vagina displays marked estrogen effect and shows only eosinophilic karyopyknotic cells, the administration of testosterone produces with increasing dosage the disappearance first of the eosinophilia, and secondly of the karyopyknosis because the estrogenic threshold level for the eosinophilia is higher than that of pyknosis. Later, the intermediate cells become predominant, but may show a certain degree of nuclear retraction as evidence of a persistent estrogenic effect. However, if the testosterone is given in a complete anti-estrogenic dosage, one can see the typical androgenic smear type, and this type only can be considered as typical or specific for androgenic activity. The androgenic smear type appears physiologically only after the menopause or castration in nearly 8% of all women and is due to a compensatory and transitory hyperactivity of the adrenal cortex. It appears exclusively if the hormonal state of the woman is completely predominated by androgens.

The androgenic smear indicates only that there exists a predominance of androgens in the patient. However, as this is only the end-result of all hormonal stimulations acting on the vaginal epithelium, we cannot determine the degree of androgen production or the absence of estrogen production. On the other hand, there may exist in some cases a high androgenic level, endogenous or exogenous, without any cytological evidence, if the patient produces at the same time estrogens in amounts sufficient to neutralize the typical androgenic reactions in the vaginal epithelium. (See figure 1)

Bibliography:

The references concerning the androgenic vaginal reactions are too numerous for complete listing at this place. They can be requested from the author or found in the following papers:

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CLAUDE GOMPEL
Brussels, Belgium

Since the description of the androgenic cell type, many discussions have taken place concerning the validity of this concept.

The cytologic pattern of this cellular type consists of a parabasal or intermediate cyanophilic cell with a large, regular hypochromatic nucleus and a pale often vacuolated cytoplasm (Figures 1,2,3). The size of the nucleus ranges between 7 and 12 microns approximately. Binucleation is not rare. Studies of the deoxyribonucleic acid content of nuclei by the histophotometric method of Lison showed us a relatively low deoxyribonucleic acid content of "androgenic cell type" nuclei. The cytoplasmic vacuoles when present are single or multiple; they vary in size and shape but are rather poorly defined. The cytoplasm of intermediate cells contain glycogen.

This very particular cell type is, in our opinion, suggestive of an androgenic stimulation but is not pathognomonic of male hormone-stimulation.

The "androgenic cell type" will appear in the vaginal cytology when there is a rather "pure" androgenic stimulation either spontaneous or therapeutical. These cells will be particularly numerous during the growth stimulation period which follows the administration of androgens in atrophic vaginal mucosae. They won't be present when the androgens are "neutralized" by estrogens for example. In this case, the vaginal smear will reflect the sum of the various hormonal stimuli.

The cytological mechanism of apparition of the "androgenic cell type" is not known. Androgens possess a variety of biological activities which involve growth stimulation, variations of specific enzyme concentrations, nitrogen retaining properties, increased vascularity and modifications of the muscle mass. Growth stimulation and variations of enzyme concentrations may help explain the nuclear variations of size and structure.

A distinction should be clearly made between the "androgenic cell type" and the "androgenic smear type." Other elements such as vaginal pH, the bacterial flora and the absence of eosinophilic pycnotic cells and leucocytes are also characteristic of androgenic stimulation. The absence of androgenic cells does not mean there is no male hormone activity and therefore the presence of that cell type cannot be used as a quantitative test of androgenic activity.

On the other way, we have never encountered androgenic cells in circumstances when androgenic activity could be ruled out. For example, we have never found androgenic cells after bilateral adrenalectomy and oophorectomy.

To conclude, we think that the existence of an androgenic cell type has been undoubtedly recognized but it is not the only diagnostic means of estimating androgenic activity.

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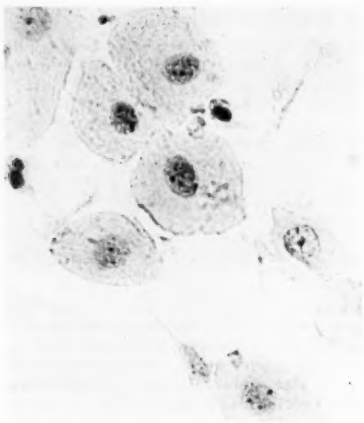


Fig. 1. (By J. Paul Pundel) Androgenic cell type.
Patient, 38 years, castrated by Wertheim hysterectomy for cancer, treated by 6 pellets of 100 mg testosterone. Smear taken 4 weeks later. Vaginal biopsy showed complete atrophy prior to treatment, and marked hyperplasia of the outer basal and intermediate layer, at the time when this smear was taken.

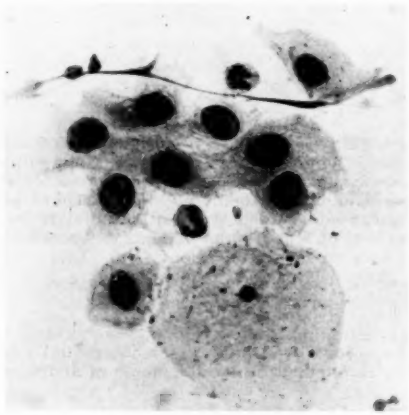
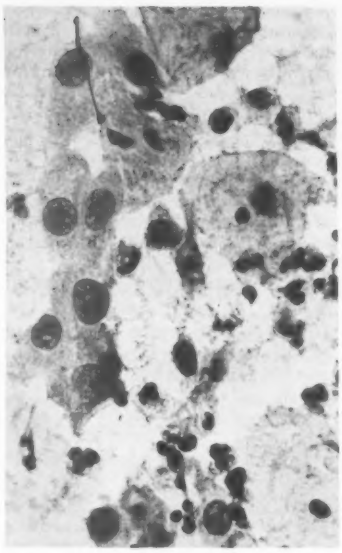


Fig. 1. (By Claude Gompel) Androgenic cell type.



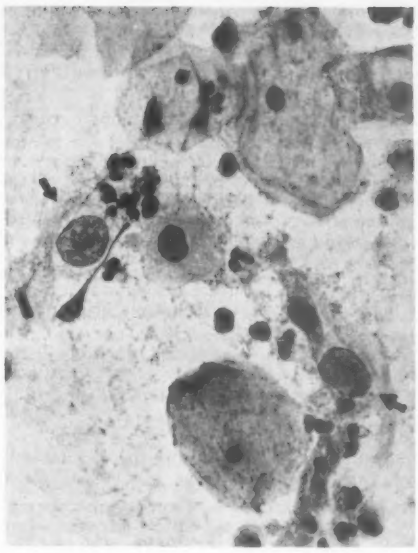


Fig. 2. (By Claude Gompel) Androgenic cell type. Fig. 3. (By Claude Gompel) Androgenic cell type.

DISCUSSION:

JOHN A. FINKBEINER * (by invitation), New York, New York, U.S.A.:

Pundel has properly indicated the importance of the total vaginal cell pattern in the interpretation of the so-called "androgenic cell type" and has stressed inability of the vaginal mucosa to act as a qualitative bio-assay analyser. As a target and organ, the vaginal mucosa, as expressed by exfoliative cytology, can only reflect the summation of various stimuli (or lack of them). In this sense, one can fairly consistently find in vaginal smears of patients with either endogenous or exogenous androgen of adequate dosage a characteristic cell type: this cell originates in the intermediate or outer deep layers, has pale basophilic cytoplasm with a large vesicular nucleus but characteristically has a perinuclear "halo" of fairly coarse bronze-colored granules (Papanicolaou EA 36 stain) which by Best-Carmine stain are positive for glycogen. While these granules are much coarser than those seen under ordinary circumstances, it is only by consideration of the entire vaginal cell spectrum that an androgenic effect may be suspected.

Some women with advanced mammary cancer who have received 50-100 mg of testosterone proprionate intramuscularly three times weekly for at least 4-6 weeks may show the typical pattern described by Dr. Pundel. Other patients may show a completely atrophic smear with no evidence of parabasal and intermediate cell proliferation and no "androgenic cell types."

Although the "androgenic cell" pattern may be seen frequently in patients receiving various androgens, there is no proof that the marked proliferation of the parabasal and intermediate layers are due solely to androgen, and not some metabolic by-product, such as estrogen.

Until further evidence is available, this question is open to speculation.

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HERBERT RAUSCHER * (by invitation), Vienna, Austria:

By and large, most authors agree: 1) that administration of androgens to women with atrophic cell type may produce proliferative effects on the vaginal epithelium; 2) it is almost generally accepted that this proliferative effect is restricted to the appearance of intermediate cells; 3) we know that all sex hormones may evoke the development of intermediate cells. Therefore, in our opinion, a smear pattern, in which the highest proliferative degree consists of elements from the intermediate layer cannot be considered specific for the activity of a particular hormone. According to Wied the "androgenic proliferative type" is characterized by a smear pattern consisting of intermediate and superficial non-cornified elements exhibiting slight crowding and moderate folding. Pundel considers typical for the "androgenic cell type," a smear pattern in which the singular cells are exclusively of the parabasal or intermediate type, desquamated generally in more single and flat elements and—by the Shorr technique —characterized by pale basophilic staining of the cytoplasm and displaying a relatively large nucleus. He points out, however, that by considering a single intermediate cell in a smear, it is not possible to make an accurate diagnosis. More important for the diagnosis of androgen effect in his opinion is the

general pattern. Years ago, we studied the effect of progesterone and testosterone in surgically castrated women with atrophic smear type. Without knowledge of the hormone given, we were not able to distinguish between progesterone and androgen-induced proliferation though we considered for the diagnosis both singular elements and the general pattern. Since this time we believe along with other authors, notably Rakoff, that by means of smear examination we cannot scientifically ascertain if a vaginal epithelium is under the influence of androgens. This does not exclude evaluation of the degree and duration of androgen effect if the basic hormonal situation is fixed as in surgically castrated women with atrophic cell type. In women with intact ovarian function, the evaluation of administered androgens seems to us to be much more difficult. In those cases we must restrict ourselves more or less to evaluating regression of the signs of estrogen activity. We would like to point out that the classification of a smear pattern in terms of estrogen activity in our opinion seems justified only if cells specific for estrogen stimulation undoubtedly are present. If they are absent, one could better speak of degrees of proliferation. We regard this especially advisable for the smear examination of women in the menopause in order to avoid misleading interpretations concerning the correlation between carcinoma of corpus uteri and estrogen activity. We consider the results published by Stoll a good demonstration of this correlation. They are almost identical with those of our own material.

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GUILLERMO TERZANO, Buenos Aires, Argentina:

Androgens produce changes in the vaginal epithelium depending on the dosage, the age of the patient, the day of the menstrual cycle, etc.

After the administration of androgens, normal epithelium may become atrophic and atrophic epithelium may show proliferation. After androgen administration the nuclei in intermediate and superficial type of cells show less pyknosis, are pale, and look rather vesicular. However, it is difficult from one smear (not knowing the previous treatment) to call it an "androgenic cell type," and be sure whether it is due to estrogen deficiency or due to androgens.

GEORGE L. WIED, Chicago, Illinois, U.S.A.:

Just for our general information, and by no means as criticism, I would like to ask Dr. Gompel if he would be kind enough to tell us into which cytological criteria some of the cells in his three excellent photomicrographs differ from histiocytes.

CLOSING REMARKS:

J. PAUL PUNDEL:

I am glad to see that there exists general agreement about the main biological effects of androgens upon the vaginal epithelium and smear and that there remain only minor differences of opinion or interpretation.

Gompel has presented a short, very interesting summary of the problem, and I agree completely with his conclusions. I also believe that the proliferation induced by androgens could only be the result of the markedly increased vascularity of the vaginal stroma which, in some cases, can take on very marked proportions. Hence, it could be possible that androgenic proliferation of the vaginal epithelium is only a manifestation of its basic growth producing potential as demonstrated in other tissues or in the healing of wounds.

I thank Rauscher for his critical discussion showing the weak points of the androgen evaluation by the vaginal smear and for his conclusions, important for practical purposes. There exist, however, some differences between the findings of Rauscher and my own material but they are minor and seem to be essentially a question of interpretation, staining technique or androgenic substances used. As I have shown elsewhere, some commercial preparations of testosterone propionate can produce a more or less secondary progestational effect.

To Finkbeiner, I answer that the appearance of the androgenic smear type is not a result of a metabolic by-product since local androgen administration induces the same vaginal epithelial response. Finkbeiner and Terzano speak about the occurrence of epithelial atrophy after androgen administration, while other cytologists and myself have never observed such reactions. To clear up this problem, the exchange of smears would be very helpful, especially if smears with atrophic reactions were confirmed by vaginal biopsies.

Summarizing, I believe that the vaginal smear is an important aid for endocrinological diagnosis, but one should not forget that vaginal smears consider only one target. For a correct diagnosis, we must study the complete hormonal state which is only possible if one assembles the results of all possible tests, such as vaginal smears, endometrial biopsies, hormonal excretion studies on the urine or blood, etc. In androgen evaluation the ketosteroids in the urine will remain the basic test and the vaginal smear is only a secondary aid, but a very helpful one, if correctly used and interpreted.

CLAUDE GOMPEL:

In answer to Wied's question I would say that I believe these cells can be differentiated from histiocytes for the following reasons: 1) the larger size of the nuclei, 2) the chromatin pattern which is much finer than in histiocytes, 3) the cytoplasm is not foamy as in histiocytes (the vacuoles even when they are small and multiple have not the uniform disposition of histiocytic microvacuoles). This aspect is not well demonstrated, I agree, in the photomicrographs. 4) finally the cellular limits are very marked in epithelial cells as compared with histiocytes, and 5) the size of the epithelial cells is bigger than histiocytes.

Reviewing the entire discussions I would like to point out that the various opinions presented are actually rather similar in their general pattern. I believe we may summarize these opinions by saying that:

1. Administration of androgens to women produce modifications of the vaginal smear which show in some cases a typical pattern called "androgenic smear type." It will only be present when the hormonal stimulation is due to androgens.

2. In all other cases, the vaginal smear will reflect the combinations of various hormonal stimuli which may exist.

The question stated in the title of this discussion has not been answered. Does an androgenic cell type exist? We would all agree that there is in some instances an androgenic smear pattern. But can we recognize a typical androgenic cell with definite nuclear modifications? This problem will give us the pleasure of more interesting discussions.

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EFFECT OF ADMINISTERED ANDROGENS ON THE VAGINAL EPITHELIUM OF WOMEN EXHIBITING THE ATROPHIC MENOPAUSAL CELL TYPE

H. WERNER BOSCHANN* (by invitation)
Berlin, Germany

In approximately 75 out of 100 women with an atrophic menopausal cell type the vaginal epithelium is proliferated by administered androgens to the intermediate or superficial non-cornified cell layers (with vesicular nuclei). There is generally a latent period of 2-3 days following administration after which the first signs of epithelial proliferation may be observed. Between the sub-threshold dosage of androgens and the threshold dosage which induces proliferation, there is a distinct dosage which does not induce proliferation, but which induces increased exfoliation of parabasal cells. The degenerative changes in the parabasal cells which are often observed in atrophic menopausal cell types disappear after administration of this sub-proliferative dosage, and the picture changes from scanty exfoliation to mass exfoliation. The cells now are generally well-preserved and exhibit distinct cytoplasmic membranes. The usual anisokaryosis of the atrophic cells decreases, but will disappear only after administration of the threshold dosage of androgens which induces a true proliferation. The administration of a threshold dosage induces in the majority of women with an atrophic menopausal cell type the following effects: after 2 to 3 days the parabasal cells gradually disappear and are replaced by glycogen-containing intermediate and superficial non-cornified cells (with vesicular nuclei); the height of proliferation is generally reached at the 6th or 7th day after the administration, i.e., 1 to 2 days later than the maximal proliferation reached after administration of estrogens. At the height of androgen-induced proliferation one may observe up to 20% superficial cells with pyknotic nuclei. The height of proliferation differs usually with the type of androgens administered. (Table I)

Milligram for milligram administered, nortestosterone enanthate and dehydroandrosterone enanthate exhibited less marked androgen activity on the vaginal epithelium than the other androgen compounds tested. Even at the height of induced proliferation, rarely do the parabasal cells disappear completely.

More marked androgen activity was evidenced by the following substances: Methyltestosterone, testosterone propionate, testosterone isobutyrate, testosterone enanthate and testosterone cyclopentylpropionate. After administration of these compounds one could observe the typical picture of "androgenic proliferation"; the parabasal cells disappear completely and glycogen-containing intermediate and superficial non-cornified cells appear. Rarely one may find superficial cells, with pyknotic nuclei; however the karyopyknotic index remained, in all cases, below 20%.

The most marked proliferative effect was observed after administration of methylandrostendiol and ethinylandrostendiol. After administration of the latter substance, the induced effect was similar to an estrogenic effect: the karyopyknotic index rose up to 70%. (Table II)

Within the range of individual variations, the higher the proliferation of the epithelium by the androgenic substances, the longer will be the time required for the epithelium to regress to the initial stage of epithelial atrophy.

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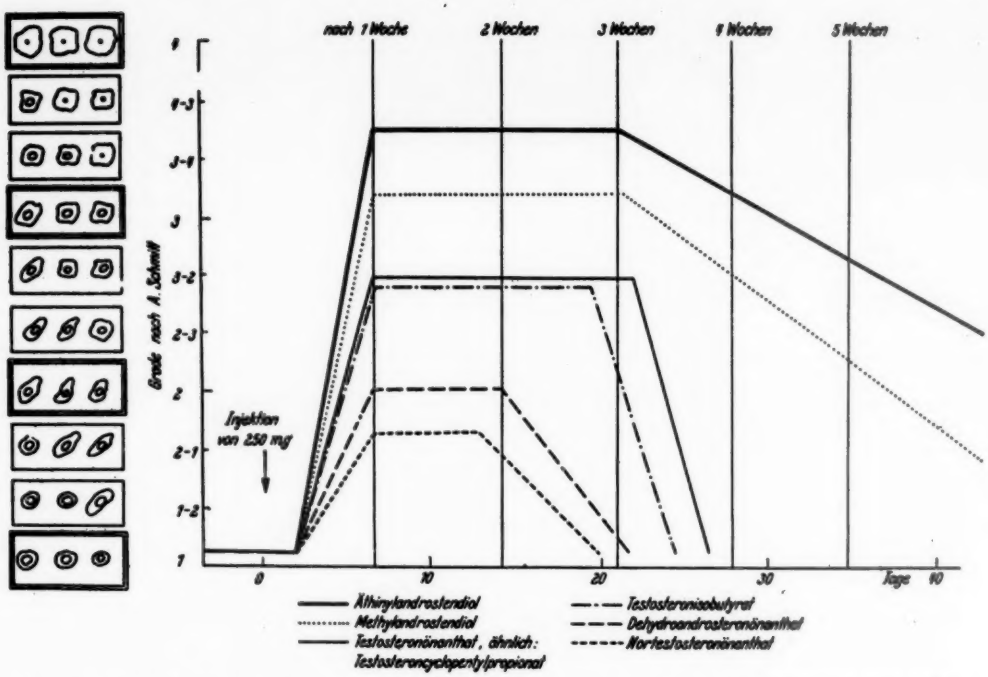

Table I. Height of proliferation and average time of duration after injection of 250 mg plus ester[1].

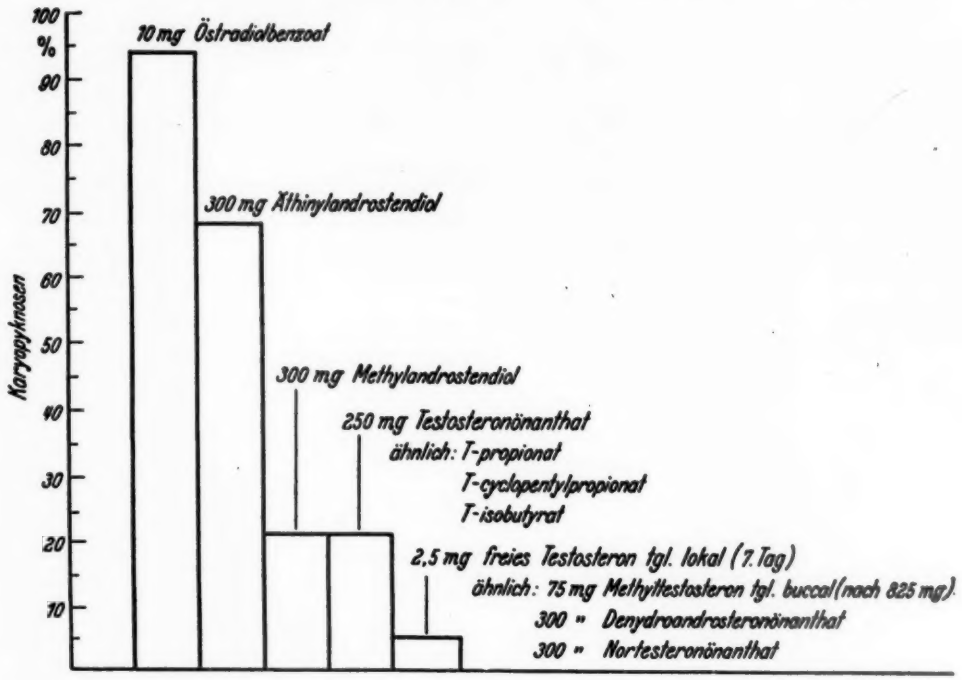

Table II. Karyopyknotic indices of various tested androgens as compared with estradiol benzoate (maximal values)[1].

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DISCUSSION:

JOHN A. FINKBEINER* (by invitation), New York, New York, U.S.A.:

Boschann has stated that after the administration of Ethinyl androstendiol, the karyopyknotic index rose to 70%. Unfortunately, the route and duration of administration and number of cases observed were not stated. However, one can speculate that this degree of differentiation of the vaginal smear pattern probably indicates conversion to estrogenic substances, manifest as an estrogenic effect rather than "similar to estrogenic effect" as he has stated. In fact, if one assumes that the karyopyknotic index is representative of differentiation, a manifestation of estrogenic activity, any shift in the vaginal smear spectrum, as indicated in the first four compounds in Table I, can be interpreted as possibly indicating conversion of androgen to estrogen.

It is well documented that normal men and women, castrated men and women, and castrated and adrenalectomized women can convert administered androgen to estrogen. Since proliferation and differentiation are known effects of estrogen activity, all the effects presented in Table I can be explained by this mechanism. It still remains to be established that androgens, per se, rather than a metabolic by-product, can cause proliferation, let alone differentiation of the cells of the vaginal mucosa.

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J. PAUL PUNDEL, Luxembourg, Luxembourg:

For more detailed conclusions, the author's original paper (1) should be read. The conclusions of Boschann are very interesting and based upon very careful studies. They confirm and complete in general my own findings. Some differences could be explained entirely by the use of a different terminology and different criteria for the gradation of hormonal effects and the pyknotic index. I agree with Erica Wachtel that the evaluation of androgens by referring only to proliferative reactions of the estrogenic type is not entirely correct because the androgens can produce some more or less specific reactions which can not be integrated in the scheme of Schmitt (11).

The author mentions the appearance of superficial cells and pyknotic nuclei after administration of androgens, but this conclusion should not be accepted as typical for all androgens. The superficial and especially the pyknotic cells appear only if estrogenic stimulation is present. The tables shown by Boschann demonstrate that these reactions (superficial cells with pyknosis) appear in appreciable degree only after the administration of particular androgens such as ethinyl-androstenediol, methyl-androstenediol, androstenediol depropionate, methyl-androstanolone, etc.) (2-10) characterized by very low virilization potential, are steroids with a more complex action in which one may find criteria of estrogenic, progestational and androgenic effects. The degree of each effect can vary from one compound to the other and is influenced by the hormonal state of the patient. Even some esters of testosterone with long action, in addition to methyl-testosterone can produce in some cases a more progesterone-like action in the vagina and on the endometrium. In view of the diagrams of Boschann and my own observations, I would conclude that the number of superficial cells and the degree of pyknosis after administration of a new androgen is in inverse proportion to the degree of typical androgenic activity. Pure testosterone, and testosterone acetate or propionate, have the most marked androgenic effect, while other so-called androgens result in more or less complex reactions. This confirms, by vaginal cytology, the paradoxical clinical reactions which these steroids, especially ethinyl-androstenediol and androstenediol dipropionate create in the treatment of hyperestrogenic disorders in the female.

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A. E. RAKOFF, Philadelphia, Pennsylvania, U.S.A.:

McCahey and I reported on "estrogenic effects of testosterone propionate on the vaginal epithelium and uterus of the castrate rat in 1943 (J. Urology 42:372, 1939), noting that with relatively large doses not only proliferation but also cornification of the vaginal epithelium could be produced. We too have noted that in the postmenopausal woman proliferation of the vaginal epithelium can be produced with relatively small doses of androgens whereas large doses given by parenteral injection do not regularly produce

proliferation as noted on vaginal biopsies (Proceedings of the Conference on Problems of Human Fertility, p. 111, 1943). The latter observations would not support the view that the vaginal cytology response after estrogen administration results solely from metabolic conversion of the androgen to an estrogen.

JOSE R. DEL SOL* (by invitation), Madrid, Spain:

Boschann mentions the fact that cytolysis occurs after administration of androgens in some instances. If I understand correctly, Boschann means the kind of cytolysis which is due to Bacillus vaginalis Döderlein (1,2). I wonder if Dr. Boschann would tell us if he believes that this cytolytic effect is in any way directly connected with the administration of androgens. I would also like Dr. Boschann to comment on a paper by Nieburgs and Greenblatt (3) for which I could not find any confirmation in the literature.

I believe that the induced cytolysis after androgen administration is not directly connected with the effect of androgens, and I base this opinion on the fact that one often observes this type of "bacterial cytolysis" (1) in the luteal phase of the normal menstrual cycle, during normal pregnancy and during long-lasting estrogen therapy (4).

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GUILLERMO TERZANO, Buenos Aires, Argentina:

Most of the atrophic menopausal vaginal smears show evidence of proliferation after the administration of androgens. Even with an amount of testosterone propionate as small as 15 mgs., proliferation becomes evident in smears.

In castrated women we did not observe signs of proliferation until the patient had received 80 mgs. in a period of 20 days.

ROBERT WENNER* (by invitation), Basel, Switzerland:

The superb examinations of Boschann which demonstrate the different effects of the known androgen substances on the vaginal epithelium of the climacteric woman with an atrophic smear type, force us to concur that these proliferations are androgen-induced and not an estrogen effect. This seems especially true since the proliferative effect is not proportional to the androgen or virilizing effect of the different substances. One fact, namely that only about 75% of the women with an atrophic type react with such a proliferation, is not explained clearly enough by the author. This makes it mandatory that we judge the effect of a hormone only after examination of a great number of patients and testing in each one all the androgenic substances. In this way only can we make valid comparisons. Our examinations, together with Mrs. Cohen-Fraenkel, have shown that in an atrophic smear type the androgenic hormone usually produces a proliferation, but that in a few cases there may be no effect at all.

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CLOSING REMARKS:

H. WERNER BOSCHANN:

In answer to Finkbeiner's question I would like to say that ethinylandrostendiol was administered as the caproate, enanthate and butyrate, in single intramuscular injections. The same dosage of each substance was injected into each of three patients. When the threshold dosage was determined we injected this dosage into 7 patients, and later a dosage slightly above and a dosage slightly below the threshold dosage. With this procedure we obtained values on 10 patients concerning these three important dosages. These and other details are described in the publication cited in my above paper. Also in answer to Finkbeiner, I would like to say that my opinion that androgens may, per se, induce proliferation is strongly supported: e.g., by the fact that androgens are approximately ten times more effective upon local (vaginal) administration compared with parenteral administration, unless someone would believe that every individual vaginal epithelial cell has the ability to convert androgens to estrogens.

I would like to thank Dr. Pundel for his remarks which reflect his great experience in the matter. The variable response of the vaginal epithelial cells leaves some doubt, however, whether one should speak of a genuine "androgenic effect." Each of the tested androgens showed a special behavior the sum of which produces a rather wide spectrum. This wide spectrum of induced effects after administration of various

androgens ranges in its extreme to such a high degree of proliferation that it is superimposed on the spectrum of the estrogen effects. Testosterone administration results in an intermediate type of induced epithelial change within the androgenic spectrum, and may be considered characteristic for the entire group.

I am in full agreement with the findings of Rakoff.

In answer to Del Sol's question I would like to say that one may find the presence of this particular type of cytolysis ("bacterial cytolysis" of Wied) if there are enough intermediate or superficial non-cornified cells present which contain glycogen. The bacterial cytolysis occurs whether this particular height of proliferation was induced by androgens, estrogens or progestogens, endogenous or exogenous hormones. Local administration of antibiotic drugs will inhibit in every case this type of cytolysis. In answer to Del Sol's second question I would say that one will have to re-evaluate and reconsider the specificity of the smear types reported by Nieburgs and Greenblatt according to our new findings.

The observations by Terzano are in agreement with our findings, that there are many individual variations in response especially after administration of androgens.

In answer to Wenner I would say that the differences in response after androgen administration may be due (among other things) to differences in the absorption. I would like to refer also to my paper in this issue of threshold dosages of various androgens, where I could show that in some cases there was no response to orally administered androgens but there was a definite response upon local administration.

Summarizing, one may say that the initially atrophic vaginal epithelium will be changed in the overwhelming majority of cases to an intermediate type of proliferation after administration of androgens if a suitable type of administration, an adequate dosage and an adequate time of administration is provided. The various androgens may induce different heights of proliferation. Certain androgens may even induce a type of proliferation which is usually observed after estrogen administration. Estrogenic metabolites of the androgens may be responsible for this effect. However, the superior response to local administration of androgens leads one to believe that androgens have the ability per se to induce epithelial proliferation.

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EFFECT OF ADMINISTERED ANDROGENS ON THE VAGINAL EPITHELIUM OF WOMEN EXHIBITING THE NON-ATROPHIC MENOPAUSAL CELL TYPE

GEORGE L. WIED
Chicago, Illinois, U.S.A.

The term non-atrophic menopausal cell type is used to define a smear type observed in menopausal women who show a predominance of folded intermediate or superficial non-cornified cells usually in the presence of a healthy vaginal flora, and with few or no leukocytes. The folding and crowding of these cells is less marked than that usually observed in the luteal phase of the cycle or that seen following progestational therapy.

This non-atrophic menopausal cell type may persist physiologically for many years after cessation of menstruation, or after castration. We have many patients on record who exhibit this type of smear more than 20 years after a normal or surgical menopause.

Unlike the patient with an atrophic menopausal type, the menopausal woman, exhibiting the non-atrophic menopausal cell type, will usually show very little, if any, change in epithelial proliferation after administration of androgens. The result of androgen administration in these patients then is for all practical purposes nil.

A few cases may show a minimal increase in the height of cellular proliferation, but never to the flat cornified superficial cells (with acidophilia and pyknotic nuclei). On the other hand, a few cases may exhibit slight epithelial regression following androgen administration. However, in no case under our observation have we observed complete epithelial atrophy to occur as the result of single androgen administration when initially the case showed a non-atrophic menopausal smear type.

The vast majority of the cases will show no changes at all. This fact is one reason why we assume that the non-atrophic menopausal cell type is a result of predominantly androgenic physiological stimulation by the adrenals.

DISCUSSION:

H. WERNER BOSCHANN* (by invitation), Berlin, Germany:

I agree with the findings of Wied on the basis of our own series of experiments. The induced changes in the vaginal epithelial cells of the non-atrophic menopausal type are non-characteristic and cannot be distinguished from spontaneously occurring slight variations in the height of proliferation. The degree of changes depends, however, on the type of androgens used for these tests. Androgens with an additional estrogenic side effect, such as ethinylandrostendiol, or actual mixtures of androgens and estrogens with a predominant estrogenic portion may certainly also induce proliferation and an increase of the karyopyknotic

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index even if the non-atrophic menopausal type was the initial cell type. Women exhibiting the non-atrophic menopausal cell type, therefore, cannot be used for testing androgens. They may be used, however, in tests which are done with the purpose of determining if a particular androgen has also an estrogenic activity. These patients may also be used in tests to determine if the androgenic portion of an administered mixture of estrogens and androgens was supplied in sufficient quantity to block the proliferative effect of the estrogenic portion on the vaginal epithelium and—as experience shows—also on the endometrium.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

The findings of Wied are identical to my own. I have never observed a typical vaginal atrophy as the result of androgen treatment in young or old women. I also believe that the persistence of non-atrophic smear types long after the menopause depends for the most part on the activity of the adrenal cortex and not of the ovaries, since the same vaginal reactions appear and persist in surgically castrated women. In general, testosterone propionate produces no appreciable change in these smears if we consider only the degree of proliferation or differentiation; but, in many cases, the morphology of the smear changes. The cells are more isolated and the folding becomes less accentuated. I believe, therefore, that these smear types are the result of adrenocortical influence. But we must not forget that the adrenals, as proven in castrated women, can show a multihormonal activity by secreting androgens, estrogens, and/or progesterone-like substances. This may explain why we find the typical pure androgenic smear type only in 6-8 percent of these women, while in general the smears show a more complex pattern, like that appearing after the administration of desoxycorticosterone, cortisone, A.C.T.H., and mixtures of testosterone, estrogens, and progesterone.

GUILLERMO TERZANO, Buenos Aires, Argentina:

If we accept that proliferation of the vaginal epithelium in old women (smears of the non-atrophic menopausal type) is induced by androgens, as Wied indicates in his paper on the effect of physiological sex hormones in patients with inactive ovaries, it becomes logical to explain why the vaginal epithelium behaves in a different way in these cases than when there is low estrogenic level.

CLOSING REMARKS:

GEORGE L. WIED:

I would like to thank Drs. Boschann, Pundel and Terzano for their comments.

Drs. Boschann and Pundel have very excellently supplemented the discussion on this subject. I agree with Boschann that the patient with the non-atrophic menopausal cell type is not a good test subject for proliferative androgen tests. However, one may use these patients very well for the so-called regression tests, i.e., first administer estrogens and then simultaneously androgens, to test the duration and efficiency of the administered androgens on the vaginal epithelium.

I must admit that I may have been guilty of oversimplification in the past by referring to the non-atrophic menopausal cell type indiscriminately as the "androgenic proliferative type." I agree that one might better consider these non-atrophic cell types as stimulated predominantly by adrenal sex steroids. However, I would like to point out that I modified the originally used "androgenic proliferative type" (Aerztliche Wschr. 7:844-849, 1952) later to "adrenal proliferative type" (in "Gynaekologische Zytologie," pp. 24-43, Theodor Steinkopff, Dresden, 1954).

It was my impression that one could attribute the physiological proliferation in postmenopausal years or in surgical castrates chiefly to the androgens of the adrenals. As androgens act considerably better upon local administration than upon parenteral administration one may assume that androgens per se can induce proliferation and that they are not converted entirely to estrogen-like substances as some may believe. Androgen administration in patients with an initially atrophic menopausal cell type often induces a cell type which cannot be differentiated from the cell types of the physiological non-atrophic menopausal smears.

I believe that we actually would have very little disagreement on this particular subject, if we would use a term for all these intermediate types of proliferation which is purely cytological and does not imply a clinical or hormonal diagnosis.

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THE EFFECT OF ADMINISTERED ANDROGENS IN NORMALLY MENSTRUATING WOMEN

A. E. RAKOFF
Philadelphia, Pennsylvania, U.S.A.

Androgens given to normally menstruating women inhibit the secretion of the ovarian hormones by depressing pituitary gonadotrophic function, the degree of ovarian suppression depending upon the dosage, the type of androgen used, the route and frequency of administration, and also the time of the cycle in which it is given. The selection of these factors depend upon the desired therapeutic use; whether suppression of the complete cycle is desired, or inhibition of ovulation, or depression of the estrogen and progesterone effects in the

corpus luteum phase. To some degree the effect of the administered androgen can be gauged by the effects upon the vaginal epithelium by means of vaginal cytology smears. Although the cytology picture in these patients reflects primarily the effect on the patient's ovarian function, it is also influenced by several additional factors including a direct "neutralizing" effect of androgen on the estrogenic action of the vaginal epithelium, the conversion of the androgen to metabolites having some estrogenic activity, and a proliferating effect of the androgen on the basal and intermediate layers of the vagina. In the normally menstruating woman these additional factors exert a significant modifying influence only when the androgen dosage is relatively high.

Testosterone propionate given in a dosage of 25 to 50 mg., three times weekly, by injection for the purpose of inhibiting menstruation will prevent the increasing cornification of the follicular phase within three to four days, followed in an additional three to four days by increased desquamation, folding of cells, and the appearance of intermediate layer cells. Continued administration of testosterone in this dosage (300 to 600 mg. a month) is associated with the appearance of parabasal cells and an atrophic type of smear by the third to fourth week of treatment. Once established, the atrophic type of smear continues as long as therapy is maintained and does not usually progress to the "androgenic" type of smear sometimes seen in castrates on androgen therapy, characterized by pale staining intermediate type cells.

If testosterone propionate is given briefly for the inhibition of ovulation in a dosage of 50 mg. daily for three days starting in the late follicular phase, a regressive type of smear resembling that of the late corpus luteum phase occurs within three or four days after treatment is started and persists throughout the cycle. In some instances ovulation is only delayed rather than inhibited and this is indicated by return of superficial cornified cells in increasing numbers.

Smaller dosages of androgens given in the postovulatory phase for the treatment of premenstrual tension, mastalgia and dysmenorrhea, such as testosterone propionate, 10 mgs. twice a week, methyl testosterone, 10 mgs. daily, or a single injection of testosterone enanthate, 50 mgs. appear to increase the number of folded cells and mucification of the cells, but rarely cause a significant increase in intermediate cells or appearance of parabasal cells.

In summary the effect of androgens given to normally menstruating women in dosages within the usual therapeutic range produce a decrease in the usual estrogenic effects. With small dosages this is evidenced by a decrease in cornified cells and increased regressive changes resembling the corpus luteum phase. With larger dosages increasingly "atrophic" smears are noted. This author did not note cell types which could be regarded as specific for androgen in the dosage range employed.

DISCUSSION:

J. P. PUNDEL, Luxembourg, Luxembourg:

I confirm the general conclusions of the author, especially the variations in vaginal response depending upon the dosages used and the moment of the cycle during which androgens are given. In general dosages under 600 mg. per cycle and even higher do not produce the typical androgenic smear type. When administered in the second half of the cycle, small doses of androgens can accentuate the progestational reactions not only in the vaginal smear but also in the endometrium. Personally I have never seen any distinct atrophic reaction proved by vaginal biopsy after androgen treatment, but the dosages used may have been too high or too low to result in a complete mutual neutralizing effect between androgens and estrogens without the proliferating effect of the latter. I believe that the regression of the estrogenic reactions in the vaginal smear during androgenic treatment is primarily due to the neutralizing effect in the end-organs, but not to a diminution of the estrogen secretion in the ovary by pituitary depression. This second effect can be obtained only with very large doses. To support this conclusion I have two arguments: 1) The findings of fresh ruptured corpora lutea during laparotomy for uterine fibroma in patients where the effects of androgen therapy persists at the time of operation (i.e., vaginal epithelial regression from the estrogen effects, and a completely atrophic endometrium). 2) The persistence of a normal biphasic basal temperature during androgenic treatment which I have observed in several cases with persistent atrophic endometrium. The same persistence was noted by Gaudefroy (1955). I would ask the opinion of the author about this problem, and also what he means by "mucification of the cells."

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JACQUES FERIN* (by invitation), Louvain, Belgium:

The administration, in normally menstruating women, of 19-nortestosterone cyclopentylpropionate or phenylpropionate, intramuscularly, 200 to 400 mg. a month, or of 17-∝-methyl-androstane-3-one 17-B-ol, sublingually, 50 to 75 mg a day, is associated with the appearance of parabasal cells and an atrophic type of smear.

These steroids are strong anti-estrogenic substances, but exert a weak androgenic action.

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CLOSING REMARKS:

A. E. RAKOFF:

The observations of Pundel on his findings in the ovary and on the basal temperature charts are

very interesting. I must confess that my own observations do indicate pituitary gonadotrophic inhibition by androgens as indicated by FSH assay, basal temperature charts and endometrial biopsy. Perhaps dosage range and time of starting the androgen account for our differences?

I used the term mucification to indicate cytolytic changes with an increase in the mucus content of the cells as indicated by the staining reaction. Perhaps this term is not a good one since the vaginal mucification test in the rat vagina refers to certain specific early proliferative changes in the parabasal cells indicative of slight estrogen stimulation.

The data of Ferin is useful. Since the steroids he employed are weak androgens it would be of interest to know their mode of action; do they inhibit FSH or "neutralize" the effect of estrogens on the target organ?

* * *

CAN ONE TELL BY MEANS OF EXFOLIATIVE CYTOLOGY AN "ANDROGENIC CELL TYPE" FROM CELL TYPES WHICH OCCUR DURING THE LUTEAL PHASE OF MENSTRUAL CYCLE, FROM CELL TYPES WHICH OCCUR DURING NORMAL PREGNANCY, FROM THE "CROWDED MENOPAUSAL CELL TYPE," AND FINALLY FROM CELL TYPES WHICH OCCUR AS A RESULT OF ADMINISTRATION OF LOW DOSAGES OF ESTROGENS? IF YES, HOW?

J. PAUL PUNDEL
Luxembourg, Luxembourg

The androgenic cell type has a typical pattern: pale, characteristic basophilic cytoplasm, rich glycogen content and a large, pale nucleus. It appears under marked androgenic stimulation sufficiently potent to neutralize in the vaginal epithelium the typical effects of other sexual hormones, especially that of the estrogens. It is practically identical with the basophilic intermediate post-partum cell type, but the general pattern of the whole smear is very different (presence of numerous leukocytes and/or eosinophilic intermediate and parabasal cells).

If we consider only the single intermediate cell in a smear, it is not possible to distinguish with complete accuracy between the following: a) the intermediate cell of the normal menstrual cycle, b) cells influenced by low dosages of estrogens, c) the cell effect of mixtures containing androgens in small dosage, d) the cellular result from compounds with multihormonal activity. In each case, however, the general pattern of the smear permits a differential diagnosis. Furthermore, local differences in the hormonal sensitivity in some areas of the vaginal epithelium reflect the influence of androgens, estrogens and/or progestational agents on the cells. Some areas less sensitive to estrogens show the typical androgenic reaction while in other areas with a higher estrogenic sensitivity, the androgens are insufficient to induce a complete neutralizing effect. The typical mixed androgenic smears are observed in cases of adrenal tumors, and following administration of cortisone, A.C.T.H. or androstenediol dipropionate.

As stated above in my answer to the question, "Is there a physiological cell type which may be defined as androgenic cell type?" it is impossible to diagnose by the vaginal smear any specific androgenic effect, if, simultaneously, estrogens are acting in sufficient strength to neutralize the typical androgenic reactions, even though the androgens are sufficient to neutralize partially the full estrogenic effect, such as eosinophilia and complete pyknosis on the superficial cells.

DISCUSSION:

PETER STOLL, Heidelberg, Germany:

All the findings which are mentioned in the title of the paper are summarized in my reading "intermediate proliferation." Because cytology is regarded in practice as a clinical technique one may be able to differentiate such individual readings as luteal phase, pregnancy, crowded menopausal cell type from the patient's history. We request clinicians to supply information and ask questions such as:
(1) Second part of menstrual cycle according to history; does the cytological picture correspond with this history? or
(2) Pregnancy possible according to patient's history; does the smear corroborate this possibility? or
(3) 6 years postmenopausal according to history; what is the hormonal condition?

During the luteal phase one will find intermediate proliferation with a tendency towards maturation (superficial cells), usually with Bacillus vaginalis Döderlein and leukocytes present.

During pregnancy one will observe intermediate proliferation, but mostly intermediate cells. The cells take up less stain, are pale, and appear watery. There is marked crowding of cells and practically always an abundance of Bacillus vaginalis Döderlein.

In cases of crowded menopausal cell types there is generally an intermediate proliferation with a tendency towards atrophy. There are, therefore, some parabasal cells present, a mixed vaginal flora, rarely Bacillus vaginalis Döderlein, and often abundant leukocytes.

Changes in the vaginal flora may make the hormonal reading difficult or even impossible. In cases of intermediate proliferation during the menopause (crowded menopausal type), one observes that the glycogen content of the cells is lower than during the luteal phase or pregnancy. During the latter

two conditions progesterone is produced simultaneously with estrogens which may be responsible for the maintenance of the synthesis of glycogen. This may also be the reason why the luteal phase and pregnancy state provide favorable living conditions for the Bacillus vaginalis Döderlein whereas these conditions are not favorable during the menopause.

GUILLERMO TERZANO, Buenos Aires, Argentina:

I agree with Pundel that the vaginal smear is a "qualitative" test for sexual hormones. I would say that quite often it is a matter of "interpretation" whether one considers one type of cell different from another.

Only a large personal experience will permit us to distinguish minute changes in the cell, in the cytoplasm, and in the nucleus.

Even in special cases, such as castrated women or women with congenital agenesis of the ovaries, during treatment it is not easy to recognize among hypotrophic smears, which one belongs to a woman treated with estrogens and which was treated with androgens.

CLOSING REMARKS:

J. PAUL PUNDEL:

To Stoll I would point out that in my opinion, the "crowded menopausal smear type" is not a definite hormonal entity, but a collective term for smears which can appear under different hormonal stimulations. Most frequently, these types are found in a hypoestrogenic state, but they can also appear under androgenic stimulation, especially if there exists a persistence of estrogenic stimulation. These smears present apparently the same picture and a differential diagnosis is very difficult. It is possible to differentiate these types by testing the vaginal pH or the glycogen content: Androgenic smears have a high glycogen content and a low vaginal pH (4,8- 5,2), while the hypoestrogenic crowded type has a low glycogen content and relative high vaginal pH (5,5- 6,5). Under certain conditions, it is possible to produce in these androgenic crowded menopausal types a marked growth of Doederlein bacilli even with resulting cytolysis, as is possible in pregnancy or the luteal phase of the menstrual cycle. I agree with Terzano that the qualitative interpretation of the androgenic activity by vaginal smears is quite difficult without great experience, because the diagnosis is mostly based upon subjective criteria and not clear objective criteria as for estrogenic evaluation.

* * *

IF ANDROGENS INDUCE GROWTH OF THE ATROPHIC VAGINAL EPITHELIUM IS IT THEN CORRECT TO REFER TO DEGREES OF PROLIFERATION OF THE VAGINAL EPITHELIUM IN TERMS OF GRADATIONS OF ESTROGENIC ACTIVITY, SUCH AS "SLIGHT ESTROGENIC EFFECT" OR "MODERATE ESTROGENIC DEFICIENCY"?

ERICA WACHTEL
London, England, Great Britain

It is almost universally agreed that administration of testosterone to women with atrophic smear patterns has a proliferative effect on the vaginal epithelium. Comparison between pre- and post treatment smears shows a change in cell type, i.e., from the prevalence of basal cells to a prevalence of intermediate squames, characterized by a hypochromatic basophilic staining reaction of the cytoplasm and pale staining, slightly enlarged nuclei. These cells are rich in glycogen. Cornified superficial cells with pyknotic nuclei are absent and no increases in dosage provokes further proliferative activity.

In contrast to the androgenic smear type, the estrogenic smear is characterized by a prevalence of superficial cells, poor in glycogen content and a high karyopyknotic index.

In my opinion, it is, therefore, illogical to classify androgenic smear patterns in terms of estrogenic activity. Histologic investigations of the vaginal mucosa confirm the proliferative effect of testosterone administration as evidenced by an increase of the intermediary layer, but in contrast to estrogen administration there is no proliferation of the superficial layer and absence of nuclear pyknosis. The effect of androgens should be regarded as specific and assessed on its own merit without reference to "estrogenic effect."

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DISCUSSION:

JOHN A. FINKBEINER * (by invitation), New York, New York, U.S.A.:

Terminology is difficult at best, especially in a relatively new field characterized by a plethora of morphologic description and a paucity of qualitative and quantitative correlation with other methods of hormone assay. The human female has no estrus cycle, and the clear-cut alterations notable in smears of rats, guinea pigs, etc. are lacking. Since the interpretation of the human vaginal smear must necessarily depend on changes in the total vaginal cell pattern, the spectra of activity reflects alterations in proliferation differentiation, and desquamation of the vaginal cells.

This problem of semantics has been further complicated by the common usage of terms indicating maximal activity. Although the cornified cell is the dominant cell of the vaginal smear in normally menstruating women, the emphasis on cornification, karyopyknosis, or similar expressions of maximal activity fail to import the total spectra of the vaginal cell pattern and further complicate the problem, since any activity less than maximal cannot be adequately expressed.

Rakoff's suggested terminology is an attempt to express relative degrees of activity or deficiency (1). As such, the idea is commendable although entirely inadequate. When introduced, the relative degree of estrogen deficiency and activity were presented as manifestations of ovarian activity. It is now well recognized that extra-gonadal sources of estrogen exist and may exert considerable effect on the vaginal smear.

Since the administration of androgen, estrogen, or progesterone to some women in appropriate dosage causes a proliferation of the vaginal mucosa, it does not seem logical to describe all these effects in terms of estrogen activity or deficiency. However, on more careful reflection, most of these studies have been performed on patients with intact (although possibly inactive) gonads, intact adrenal glands, and unknown liver function and nutritional status.

As has been indicated elsewhere in this symposium, the vaginal mucosa is a target and organ and not a biochemical analyzer, either quantitatively and qualitatively. Various patients receiving testosterone may show several different vaginal smear patterns. It is far from proven that the proliferation of the vaginal mucosa noted after either testosterone or progesterone therapy is due to an effect of these drugs per se, especially in a patient with intact gonads and adrenals. It is much more likely that the effect of the exogenous hormone (and its by-products) plus endogenous hormones. This explains the close similarity of the vaginal cell patterns noted in one of the presentations of Pundel.

The problem of terminology certainly merits far more thought, consideration, and discussion by this Academy.

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* John A. Finkbeiner, M.D., 444 East 68th Street, New York, New York, U.S.A.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

I can only approve entirely the conclusions of Erica Wachtel.

A. E. RAKOFF, Philadelphia, Pennsylvania, U.S.A.:

I would agree with Wachtel that it would be more accurate to describe cellular patterns on a smear rather than interpret the effect in terms of estrogen. On the other hand, it would be equally inaccurate to classify a smear as an "androgenic type" since this pattern is not specific for androgens alone, nor can it be quantitated accurately in terms of androgen. Why not simply describe the cell types present to which the cytologist can then append an interpretation.

PETER STOLL, Heidelberg, Germany:

For practical purposes in cytological interpretation, we distinguish between the following types of proliferation:

1. Marked proliferation (estrogenic)
2. Intermediate proliferation (progestational, androgenic, or pregnancy)
3. Lack of proliferation (no hormonal stimuli)

For scientific studies, 100 squamous epithelial cells are counted at random and the incidence of the following types of cells determined: Basal, intermediate blue, intermediate red, superficial blue, superficial red. Utilizing this classification, the following smear types are distinguished:

1. Marked estrogenic
2. Estrogenic
3. Intermediate proliferation with tendency towards maturation
4. Intermediate proliferation
5. Intermediate proliferation with tendency to atrophy
6. Atrophy

During the menopause we speak of an androgenic type if smear types such as described above under 3, 4, and 5 are found; thus avoiding terms such as "slight estrogen deficiency." However, we do not associate readings of 3, 4, and 5 with androgenic stimulation only, since one or other might occur with the

onset or cessation of estrogenic stimulation of the vaginal epithelium. Especially during the early menopause, consideration must be given to the so-called post-menopausal cycle, where sub-threshold estrogen levels not sufficient to stimulate the endometrium, may be strong enough to be detected by means of vaginal cytology. During the menopause it is not sufficient to classify a smear as "androgenic" on the basis of a single smear examination. It is necessary that the smear type remains unchanged over a distinct period of time before giving this reading. We usually request 3 smears at intervals of 4 days each, and perhaps even this number may not be adequate.

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GUILLERMO TERZANO, Buenos Aires, Argentina:

The pattern of the smear and the appearance of the nuclei in androgenic and estrogenic smears are seen to differ somewhat with Shorr's stain and Papanicolaou's staining technique.

With reference to glycogen content, we are not aware of any remarkable difference in the androgenic and estrogenic smear types since glycogen is found in every phase of the normal menstrual cycle. Nevertheless, the glycogen index has been used in vaginal smears to assess relatively estrogen effects.

I agree with Dr. Wachtel; an androgenic smear should be described as such with a report on the state of proliferation (with a percentage of each type of cell) to indicate the androgenic pattern of the smear.

ROBERT WENNER* (by invitation), Basel, Switzerland:

I quite agree with the author that we should not consider the proliferative action of androgenic hormones as an estrogenic effect, since after application of testosterone and its derivatives the acidophilia as well as the karyopyknosis disappear. We notice only a proliferation to the intermediate cells. However, the term "androgen effect" does not seem to be suitable. Bochann, e.g., demonstrated that the various androgens produce different degrees of proliferation and that the degree of proliferation does not necessarily reflect the androgenic and virilizing capacity of the hormones. It seems to me better to describe the effect and to indicate which cell type predominates.

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CLOSING REMARKS:

ERICA WACHTEL:

Summing up the evidence given it appears to me that we are unanimously opposed to expressing androgen effects in terms of estrogen activity. We are, however, unable to produce a clearly defined, universally acceptable yardstick for measuring androgen effects in vaginal smear. Why not then, until such a yardstick is available, take Rakoff's sound advice and simply describe the cell pattern presented? Interpretation of these cell patterns will necessarily include a certain amount of speculation and imagination.

* * *

THRESHOLD DOSAGES OF VARIOUS ANDROGENS, USING THE VARIOUS METHODS OF ADMINISTRATION, NECESSARY TO STIMULATE GROWTH OF THE ATROPHIC VAGINAL EPITHELIUM

H. WERNER BOSCHANN* (by invitation)
Berlin, Germany

The parenteral administration of androgens is definitely superior to any of the other methods of administration. The greatest individual variations in response is obtained using oral and buccal administration. In order to obtain the identical proliferative effect on the vaginal epithelium, one generally needs six times the parenteral dosage for buccal administration and twelve times the parenteral dosage for oral administration. If however, the androgen is applied locally, only 1/10 of the parenteral dosage is necessary to induce a similar proliferative effect. Therefore, the local dosage is related to the parenteral, buccal and oral dosages as 1:10:60:120.

Repeated administration of small dosages of androgens daily results in a more marked proliferation than the total dosage given as a single injection. For example, the single administration of 75 mg testosterone propionate produced the same effects as a total dosage of 30 mg given over a period of 3 days, or a total dosage of 20 mg given over a period of 4 days, and, finally, 15 mg administered over a period of 6 days. However, it was impossible to induce proliferation after a single administration of an oral or buccal dosage of androgens; one could observe proliferation after repeated daily administration of small dosages. Similar results could be observed after local (vaginal) administration. The time-dosage-principle which was described by Buschbeck (2) for estrogens is also valid for androgens according to my observations. It seems that the stratum germinativum of the epithelium must be stimulated by distinct minimal dosage may

be easily achieved by local administration. However, after oral or parenteral administration only a small proportion of the administered substance seems to stimulate the vaginal epithelium. This holds true even if the androgens are injected into the connective tissue of the vagina. In this case, it seems that the substance is carried away rather rapidly through the lymphatic vessels so that this paravaginal administration results in practically the same type of proliferation as the parenteral administration.

The greatest individual variations in response are observed after oral and buccal administrations of androgens. In one case we found no epithelial response whatsoever after daily oral administrations of 375 mg methyltestosterone (total dosage of 3145 mg), whereas marked proliferation could be observed after 3 local (vaginal) administrations of 2.5 mg testosterone.

It is possible to inhibit induction by estrogens of an estrogenic type of proliferation by the simultaneous administration of 20 times higher dosage (mg per mg) of androgens. The resulting smear type is that of an "androgenic proliferation." However, if the relation is changed to the disadvantage of the androgenic portion, the estrogenic effect will gradually appear. If androgens and estrogens are supplied in relation of 10:1 only, the resulting smear type is clearly estrogenic (Table II).

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TABLE I
Table of Tested Substances and their Dosages
(Underlined Dosages Designate the Threshold Dosages)(1)

Testosterone alcoholic sol. vaginal	Testosterone (Suppositoria)		Methyltestosterone (Tablettes)			Testosterone propionate		
	vaginal: lx	vaginal: repeated	vaginal	per os	buccal	para-vaginal	i.m.:lx	i.m.: repeated
		0,25 mg						
		0.5 mg						
	1 mg	1 mg						
2.5 mg 3X	5 mg	5 mg	2 mg	2 mg		2.5 mg	2.5 mg	2.5 mg 6X
	10 mg		5 mg	5 mg		5 mg	5 mg	5 mg 4X
			10 mg	10 mg	10 mg	10 mg	10 mg	10 mg 3X
			15 mg	15 mg	15 mg			
				20 mg	20 mg		20 mg	
				25 mg			25 mg	
				30 mg				
				50 mg	50 mg		50 mg	
				75 mg	75 mg		60 mg	
				100 mg			75 mg	
				150 mg			100 mg	
				375 mg			200 mg	

Testosterone isobutyrate i.m.	Testosterone enanthate i.m.	Testosterone cyclopentyl-propionate i.m.	Dehydro-androsterone enanthate i.m.	Nortesto-sterone enanthate i.m.	Methyl-andro-stendiol i.m.	Ethinylandro-stendiol i.m.
25 mg	25 mg	25 mg			25 mg	25 mg
50 mg	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg
	100 mg	90 mg	100 mg	100 mg	100 mg	100 mg
300 mg	300 mg	300 mg	300 mg	300 mg	300 mg	300 mg

TABLE II
Karyopyknotic Indices after Administration of Estradiol Valerate
or after Administration of Estradiol Valerate
plus Testosterone Enenthate(1)

		Testosterone enanthate			
		0	50 mg	100 mg	250 mg
	0		0-5%	0-5%	0-5%
Estradiol	1.0 mg	10-30%	0-5%		
valerate	3.0 mg	30-40%	5-20%		
	4.0 mg	30-50%		0-5%	
	6.0 mg	30-60%		5-20%	
	10.0 mg	over 50%		over 50%	0-5%

J. PAUL PUNDEL, Luxembourg, Luxembourg:

First, I would congratulate the author for his very interesting, important paper and for his perserverence in compiling all this data. My own findings are practically identical concerning the threshold doses and the marked individual variations that are observed after oral administration of androgens. We must not forget that in some patients the route of hormone administration, parenteral or oral, may cause a different reaction, since it is possible that during the passage through the liver variable amounts of the hormone may be changed to compounds of different action. Therefore, it may be necessary to test every hormonal compound, in studying its specific activity, by local administration in the organ under scrutiny. (For example estrogen invokes a different cytological picture, if administered locally or orally during pregnancy.) During long-term treatment by massive doses of testosterone or estrogens I have the impression that the vaginal epithelium, and for estrogens the endometrium also show a progressive diminution in response to these hormones. The targets of the hormones become refractory to their action. Only after a prolonged interruption of treatment does the vagina recover progressively its previous normal sensitivity to the same hormone. I would be glad if Dr. Boschann would give us his personal findings in such cases.

CLOSING REMARKS:

H. WERNER BOSCHANN:

I agree with the findings of Pundel on the basis of our own experimental results. In order to maintain the same height of proliferation one has to increase gradually the hormonal dosage. The vaginal epithelium reacts here in this respect similar to the endometrium (break-through-bleeding of W.M. Allen). After long-term administration of nonphysiologically high dosages both end organs finally exhibit diminishing responsiveness.

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CAN ONE DETERMINE BY MEANS OF EXFOLIATIVE CYTOLOGY THE EFFICIENCY AND DURATION OF ANDROGENIC THERAPY?

H. WERNER BOSCHANN* (by invitation)
Berlin, Germany

The very highly proliferated vaginal epithelium usually found during the late follicular phase of the normal menstrual cycle will be suppressed in its height of proliferation by rather high androgen doses (300-500 mg per month).

The moderately proliferated vaginal epithelium, as found during secondary amenorrhea, in castrates or in the majority of the cases during normal menopause, will not exhibit any epithelial changes after administration of androgens.

The atrophic vaginal epithelium, as found in the infant or in a distinct percentage of menopausal women or during the senium will show moderate cell growth to the intermediate or superficial non-cornified cell layers following androgen administration.

From these aforementioned findings it is obvious that the testing of the efficiency and the duration of androgen therapy can be measured: 1) by assessing the effect on the disruption of the normal menstrual cycle by high dosages of androgens on the highly proliferated epithelium and 2) by assessing the proliferative effect produced by rather small dosages of androgens on the atrophic epithelium.

There are, however, the following limitations to these tests:

(1) Following oral, buccal or local administration, the epithelial changes do not express necessarily the duration of the clinical effect of the administered androgens: one may clinically observe, e.g., after buccal or oral administration, immediate improvements of the subjective symptoms while no changes are present yet in the vaginal epithelium. On the other hand, upon local (intravaginal) administration, one may find proliferative changes without any clinical improvement of the symptoms. Therefore, from the degree of proliferation following local administration, one cannot draw any conclusions as to the degree of resorption of the substance or the general clinical effectiveness of the drug.

(2) With parenteral administration, the duration of clinical efficiency of the androgenic therapy is usually longer than the duration of the epithelial proliferation.

Summarizing, one may say that exfoliative cytology does not offer a definite diagnostic test for the clinical efficiency of androgenic therapy. Exfoliative cytology offers, however, the following information:

(a) whether or not an androgenic substance is effective at all,
(b) about the latent period, the degree of androgenic effect and the duration of effect as compared with other administered androgens, and
(c) in cases where estrogens and androgens are administered simultaneously: whether or not the androgenic portion of the compound is supplied in dosage adequate to suppress the estrogenic effect, and whether or not the androgen or estrogen have compatible duration of effects.

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GEORGE L. WIED and ALICE M. DARGAN
Chicago, Illinois, U.S.A.

There is ample evidence to support the following factual observations:

(a) When an atrophic vaginal epithelium is subjected to androgen stimulation, epithelial proliferation occurs in a large majority of cases.

(b) When a highly proliferated epithelium ("estrogenic type") is subjected to androgen stimulation, a regression in proliferation results if androgen dosage is sufficient to suppress estrogenic stimulation.

In a recent study utilizing these observations, it was shown that exfoliative cytology provides us with a reliable technique for determining the efficiency and estimating the duration of effects of androgen therapy. It must be conceded at least as reliable a test of hormonal activity as urinary 17-Ketosteroid examinations and clinical observations in male castrates.

A group of surgical castrates who had had estrogenic or cyclic therapy prior to androgen administration were simultaneously treated with varied dosages of androgens and daily oral administration of 1 mg of diethylstilbestrol. Vaginal smears taken every third day were subjected to cytological evaluation.

The effects induced on the vaginal epithelium were evaluated according to the following cellular indices: (a) the karyopyknotic index, (b) the crowded cell index, and (c) the folded cell index (10). Using these cellular indices, the relative duration of effects of administered androgens was determined. Appraisals of the androgen-induced proliferation of the atrophic epithelium are possible using the same indices.

The advantage of this "regression test" (10) on the estrogen stimulated epithelium over the induced cellular "proliferation test" (7,9) on the atrophic epithelium is that it will always yield results, providing the estrogen-androgen relationship is adequate. However, the disadvantage of the "regression test" is that increased dosages of estrogens will result in an apparent prolongation of androgen activity.

The advantage of the induced cellular "proliferation test" on the atrophic epithelium over the above "regression test" is that low dosages of androgens may be sufficient in some cases to induce epithelial growth. The disadvantages are, on the other hand, that a distinct percentage of test individuals with an atrophic epithelium will not respond to administered androgens with an induced growth of the vaginal epithelium.

The above question whether or not it is possible to determine the efficiency and duration of androgenic therapy by means of exfoliative cytology may be answered conditionally in the affirmative, although it is admitted that the resulting smear types are essentially non-specific. (See figures 1 & 2).

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9. Wied, G. L. and Suchowsky, G.: Gehurts. und Fruaneheilk. 14:645-650, 1954.
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DISCUSSION:

J. PAUL PUNDEL, Luxembourg, Luxembourg:

I agree with Boschann concerning his findings and conclusion with one reservation: the subjective symptomatology is not a good criterion for testing the efficiency of androgenic or other hormonal compounds. This was shown previously by Wied (3). In my own experience, based upon more than 800 patients, treated with androgens for hyperestrogenism, uterine myomas, metastatic breast cancer and postmenopausal syndrome, objective improvement in the clinical state appears only if the vaginal cytology shows a clear androgenic reaction. Subjective improvement on the other hand may be apparent after doses of androgens which are yet ineffective on the vaginal epithelium and the endometrium. The vaginal smear therefore remains one of the best, if not the best objective test of the efficiency and duration of the hormonal action of new androgenic compounds.

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1. Pundel, J. P.: "Les frottis vaginaux endocriniens" Masson, Paris, 1952.
2. Pundel, P. P.: "Acquisitions recentes en cytologie vaginale hormonale" Masson, Paris, 1957.
3. Wied, G. L.: Aerztl. Wschr., 8:623, 1953.

A. E. RAKOFF, Philadelphia, Pensylvania, U.S.A.

Since the changes induced by androgen in the vaginal epithelium are not specific and can be mimicked in some respects by various other steroids (progesterone, desoxycorticosterone, cortisone, weak estrogens, etc.), the interpretation of effect by vaginal cytology is not secure and certainly not

specific. This is also true of other methods of evaluating the effect of androgens in women, such as inhibition of ovulation, effect on cervical mucus and on the endometrium. No one of these methods are specific for androgen but each are valuable in measuring certain effects of androgens in the human female. We have found the cytology smear useful in evaluating the relative dosage of androgen to add to estrogen in the treatment of women with postmenopausal osteoporosis.

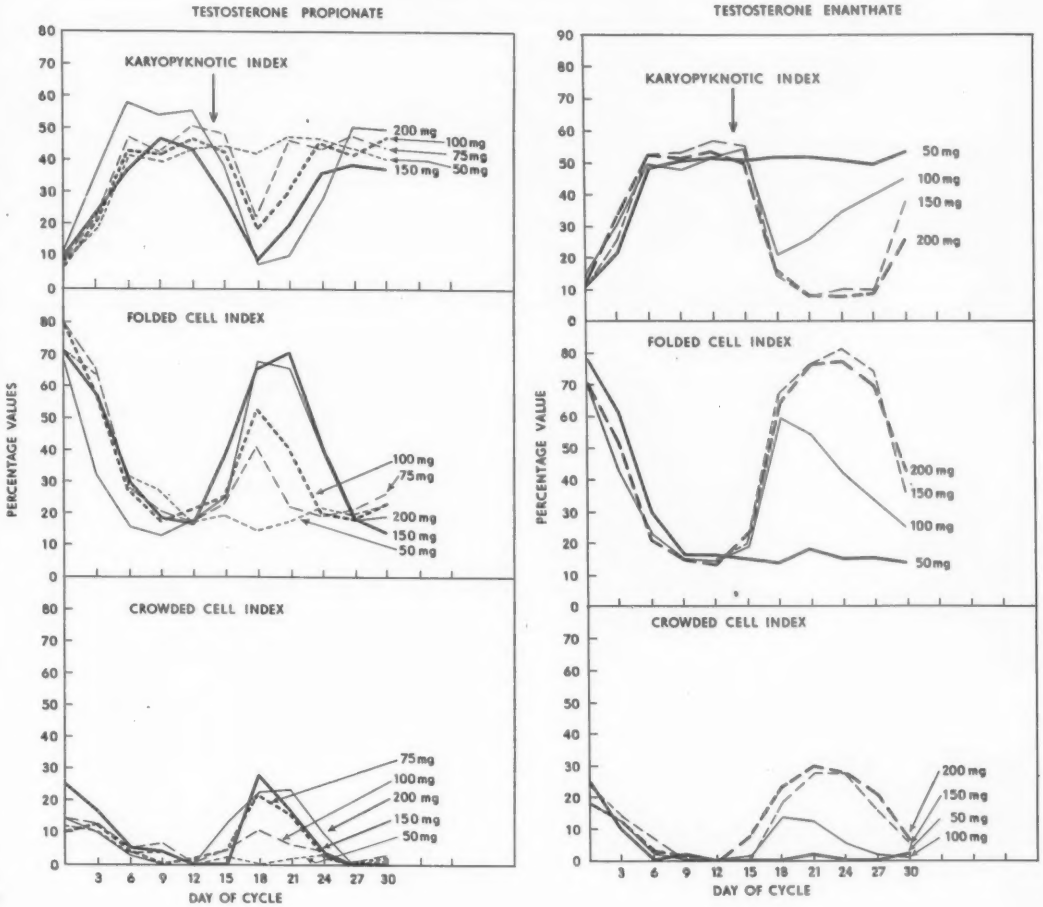

Fig. 1. (Wied) Average curves of the three cellular indices showing response of the highly proliferated vaginal epithelium of surgical castrates to various dosages of testosterone propionate administered by single injection on the 14th day of the test period. Each patient received daily 1 mg diethylstilbestrol (orally) throughout the entire test period (10).

Fig. 2. (Wied) Average curves of the three cellular indices showing response of the highly proliferated vaginal epithelium of surgical castrates to various dosages of testosterone enanthate administered by single injection on the 14th day of the test period. Each patient received daily 1 mg diethylstilbestrol (orally) throughout this entire test period (10).

CLOSING REMARKS:

H. WERNER BOSCHANN:

I am in full agreement with the findings of Pundel.

GEORGE L. WIED:

The statement by Boschann in the first paragraph of his paper that the proliferation of the vaginal

epithelium during the late follicular phase can be suppressed only by rather high dosages of androgens is correct, although we found that in our surgical castrates who received estrogens for epithelial proliferation did not need such high dosages to induce epithelial regression. This might be due to the fact that the endogenous estrogenic activity at this time of the menstrual cycle is in excess of the activity of daily 1 mg diethylstilbestrol which we administered orally in our surgical castrates.

I would also agree with the discussion of Pundel. In testing new androgenic compounds it seems to me, however, that it would be useful to use both types of testing: 1) administration of androgens to the castrate with atrophic vaginal epithelium to induce proliferation and then 2) administration of estrogens to induce marked proliferation and later simultaneous administration of androgens to induce regression in the same castrate. This makes testing procedures a little more elaborate, but—I believe—also a little more accurate.

To Rakoff's comment that the induced androgenic changes are nonspecific one can only agree. However, also nonspecific changes can be diagnostic to some degree. For testing of new androgenic compounds vaginal cytology offers certainly one of the most practical methods at this time, if one stays within the limitations of the technique. If any external factors, such as infections, Trichomonas infestations, cytolysis, leukoplakia, prolapse, or local irritations are present, the hormonal evaluation cannot be made until the factor has been treated. The considerable individual variations in response must also be taken into consideration in the evaluation.

Summarizing, I would say that even though androgen administration does not induce specific cellular changes, one can determine the efficiency and duration of androgenic therapy with a degree of accuracy which is as high as or higher than any other technique of determination known at this time.

* * *

HIRSUTISM AND VAGINAL CYTOLOGY

ERICA WACHTEL
London, England

The differentiation between familial hirsutism and hirsutism caused by endocrine dysfunction can easily be made by cytological investigations, since the former is not associated with ovarian disturbance and, therefore, does not interfere with the normal cyclical pattern. Unfortunately, there is no clear cut cytological pattern for any one of the endocrine causes of hirsutism, e.g., adrenal tumor, Cushing's and Stein-Leventhal syndromes. Smears taken from patients suffering from these disturbances show absence of cyclical changes; in addition, there are sometimes marked androgen effects present, but mixed patterns showing a combination of atrophy and androgenization or combined estrogen and androgen effects are more common. These smears are extremely difficult to interpret, since the summation of effects of various sex-hormones on the epithelium cannot confidently be analysed in detail and a differential diagnosis of the endocrine defect by cytology alone is at present practically impossible.

Bibliography:

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GUILLERMO TERZANO
Buenos Aires, Argentina

There are two main groups of hirsute women:

(1) Women with normal vaginal cytology: eutrophic vaginal smears, showing monophasis or biphasic cyclic variations to a greater or lesser extent. To this group belong those patients who have a constitutional hirsutism (hypertrichosis, cause unknown).

(2) Women with hypotrophic or atrophic smears exhibiting no cyclic changes. In this group belong women with virilism: ovarian virilism (arrhenoblastoma, virilizing tumors), pituitary virilism and adrenal virilism (hyperplasia of the adrenal cortex, adrenal tumors, adrenogenital syndrome and others).

A third group could be composed of male or female pseudohermaphrodites whose vaginal smears are of the atrophic or highly proliferated (estrogenic) type.

Apart from the cytological evidence of the presence or absence of estrogenic activity the cytological smears do not offer any specific diagnostic information. I consider myself unable to distinguish between hopoestrogenic and androgenic smears on specimens stained according to the Papanicolaou technique.

DISCUSSION:

JEAN BERGER, Basel, Switzerland:

The above mentioned classification by Terzano seems suitable. We are able to classify in these

categories all the cases observed at our clinic.

In some cases of hypertrichosis of unknown cause, we find normal cytology, biphasic cycle, but a low acidophilic level.

In congenital hirsutism, caused by a more pronounced endocrine dysfunction, no biphasic cycle could be observed. Thus, we find in the vaginal smears of 2 cases of congenital-adrenogenital syndrome before therapy, an obsolute atrophia (small basal cells). In spite of this, high phenolsteroids and 17-ketosteroids are found in the 24-hour urine.

In two other cases of virilizing adrenal tumors, as well as in two cases of virilizing adrenal diseases (Cushing), we saw an absolute atrophia in the vaginal smears. These cases could belong in class 2 of Terzano's above mentioned scheme.

We also observed two cases of "testicular feminization." The vaginal smears showed an increased estrogenic effect, in spite of a low, biologically proved, estrogenic value and diminished excretion of phenolsteroids and increased 17-ketosteroids in the 24-hour urine. (Schaumkell and Goldberg made similar observations.) Here it is of interest that in the presence of testicles an estrogenic effect can be observed in the vaginal smears, in addition to growth of the breast.

If in those cases castration is performed after puberty, the estrogenic effect in the vaginal smears dimishes gradually. Manifestations of endocrine deficiency appear and the breasts become atrophic. (Similar observations were made by Wagner, Cadig, Novak, Goldberg and others.) If the castration is done before puberty, no estrogenic effect in the smears can be seen. This leads to the conclusion, that the estrogenic effect and the growth of the breast are due to the presence of testicles. These cases could be assigned to Terzano's third class.

The vaginal smears alone do not permit a definite diagnosis. Hirsutism demands an exact clinical, endocrinological and hormone-chemical clarification.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

My own experience based upon the cytological study of 78 cases of adreno cortical hyperfunction with and without hirsutism, and a large number of patients with hirsutism with no other hormonal disturbance confirms completely the findings of Erica Wachtel. Hirsutism can be associated with a completely normal vaginal cytological picture, normal menstrual cycle and normal 17-keto-steroids. Other cases have a typical androgenic smear and a high 17-keto-steroid-output. To further confuse the issue some cases with marked adrenocortical hyperfunction, and 17-keto-steroids as high as 300 mg/24 hours, the vaginal cytology may be purely estrogenic and the endometrium may exhibit a typical glandular hyperplasia of the Swiss cheese type. The vaginal cytology gives only the summary, the end result of the different hormonal stimulations on the vaginal epithelium. This physiological law explains why in most cases it is impossible to ascertain by the vaginal smear the different hormonal actions. One can find pure estrogenic smears in typical adrenocortical hyperfunction with 17-keto-steroids as high as 100 mg/2$\frac{1}{2}$ hours, if at the same time there exists a marked estrogenic production capable of neutralizing the androgenic effect. Moreover one can observe typical androgenic smears in women with low 17-keto-steroids (4-9 mg/ 24 hours), if at the same time the production of estrogens has completely stopped. As in many cases of adrenocortical hyperfunction, the adrenals and/or the ovary can simultaneously produce androgens, estrogens and progesterone-like substances in various amounts, the vaginal smear may show in these cases variable pictures, typical androgenic, estrogenic or mixed patterns, depending on the relative amounts of the different hormones and their activity upon the vaginal epithelium. This depends furthermore, on the mutual synergetic or antagonistic activity of the different hormones. The vaginal smear is not a sensitive test that enables the separate estimation of androgenic activity in the female, but it reflects the summation of the different hormonal actions. If the vaginal smear is typically androgenic, one can conclude that androgens predominate the hormonal picture.

Bibliography:

Pundel, J. P.: "Acquisitions recentes en cytologie vaginale hormonale" Paris, 1957, Masson & Cie. (Complete references).

CLOSING REMARKS:

ERICA WACHTEL:

It is gratifying to note that we are agreed on all aspects of the problem before us, the conclusion being that, at present, cytology can distinguish only between constitutional (familial) hirsutism and hirsutism due to endocrine factors. Further differential diagnosis of the complex group of conditions in which hirsutism occurs as one of the symptoms of an endocrine syndrome is, at present, impossible.

GUILLERMO TERZANO:

I would like to thank Drs. Berger and Pundel for their very interesting discussions; they complete the picture of vaginal cytology in hirsutism.

THE NEXT SYMPOSION

LE SYMPOSION PROCHAIN - DAS NAECHSTE SYMPOSION - EL PROXIMO SYMPOSIUM

VOLUME II 1958 NUMBER 1

The next issue of the ACTA CYTOLOGICA, scheduled for publication in the Spring of 1958, will contain two main subjects for discussion as written symposia:

1. Symposion on cytological terminology.
2. Symposion on forms for clinical information and laboratory reports.

1. SYMPOSION ON CYTOLOGICAL TERMINOLOGY

A number of excellent photomicrographs of individual cells and cellular patterns will be selected. On the basis of these photomicrographs the terminology which will be used for each of the cells or cellular patterns will be considered.

The terminology approved by the majority will be the one which will be used henceforth in the ACTA CYTOLOGICA of the Academy.

The Members of the Academy are invited to submit as soon as possible any number of such photomicrographs of individual cells or cellualr patterns so that exemplary pictures may be selected and circulated.

Instructions for Photography: The photomicrographs should be prepared on glossy white photographic paper at least 5 by 7 inches (13 cm x 18 cm).

Two main types of cellular photomicrographs will be used:
1. Oil immersion photographs of individual cells: The individual cell must lie singly. Every cell (epithelial or non-epithelial which may occur in the female reproductive tract is of interest. If possible, but not necessarily, the fixed and stained cells should be photographed with a phasemicroscope in order to obtain excellent contrast and to demonstrate minute details in cytoplasm and nucleus.
2. Photographs of cellular patterns taken with 40x - objective: These photographs are mainly used to clarify the terminology of general patterns and for hormonal cytology.

Each photograph should be accompanied by the following information:
1. Name of contributor,
2. Cytological terminology suggested by the contributor,
3. Age of patient,
4. Last menstrual period of patient,
5. Clinical diagnosis,
6. Histological diagnosis, if any,
7. Model of microscope used,
8. Magnification (objective and ocular).

2. SYMPOSION ON FORMS FOR CLINICAL INFORMATION AND LABORATORY REPORTS

Members of the Academy are invited to submit their current clinical information and laboratory report forms for publication in the second part of the next issue of the ACTA CYTOLOGICA.

Based on these forms, a discussion will be held on:
1. What information must the clinician submit for proper evaluation of the cytological specimens?
2. What cytological reading (report) is routinely given to the clinician from the laboratory?

The Members are requested to submit explanatory remarks (maximum of 350 words) with each of their forms.

Deadlines for the Above Symposion:

1. The microphotographs together with the required legends must reach the Editorial Office (5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.) not later than March 1, 1958.
2. The forms for clinical information and laboratory reports together with the legends and explanatory remarks must reach the Editorial Office (5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.) not later than March 15, 1958.

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FUTURE SYMPOSIA

LES SYMPOSIONS FUTURS - ZUKUENFTIGE SYMPOSIA - SIMPOSIUM FUTUROS

VOLUME II 1958 NUMBER 2

The Written Symposion of this issue will be devoted to the discussion of three main subjects:

A. Definition, morphology, cytochemistry and diagnostic importance of spindle shaped squamoid cells (snake cells, fiber cells, spindle cells).
B. Routine staining techniques other than the Papanicolaou technique.
C. Effects of administered estrogens.

Deadlines for this Issue

1. Members of the Academy who wish to be a main speaker or suggest guest speakers on any of the topics for discussion listed below must inform the Editorial Office, not later than April 15, 1958, of their desire to actively participate.
2. The Main Papers must REACH the Editorial Office not later later than June 1, 1958.
3. The Discussions of the main papers must REACH the Editorial Office (5841 S. Maryland Ave., Chicago 37, Illinois, U.S.A.) not later than August 1, 1958.
4. The Closing Remarks of the Main Speakers must REACH the Editorial Office (5841 S. Maryland Ave., Chicago 37, Illinois, U.S.A.) not later than September 15, 1958.
5. A tentative list of proposed subjects is outlined below. The Members of the Academy may propose additional points for the list by writing to the Editorial Office (5841 S. Maryland Ave., Chicago 37, Illinois, U.S.A.) not later than March 1, 1958. Members of the Academy who suggest additional points of discussion, however, must either agree to be the Main Speaker on this particular new point or submit the name of a willing contributor.

Preliminary List of Points of Discussion:

A. DEFINITION, MORPHOLOGY, CYTOCHEMISTRY AND DIAGNOSTIC IMPORTANCE OF SPINDLE SHAPED SQUAMOID CELLS (SNAKE CELLS, FIBER CELLS, SPINDLE CELLS)

1. Definition
2. Morphology
3. Cytochemistry
4. Phasemicroscopy
5. UV-microscopy
6. Electron microscopy
7. Colpo-microscopy
8. Tissue culture
9. Animal experiments
10. Spindle cells in infections
11. Spindle cells in dysplasia
12. Spindle cells in carcinoma in situ
13. Spindle cells in invasive cervical carcinoma
14. Spindle cells in non-genital carcinomas
15. Are spindle cells suggestive of a distinct type of carcinoma or of a distinct type of maturity of cells?
16. Are spindle cells derived from the surface of the lesion?

B. ROUTINE STAINING TECHNIQUES OTHER THAN THE PAPANICOLAOU TECHNIQUE

1. May Gruenwald Giemsa (advantages and disadvantages)
2. Hematoxylin Eosin (advantages and disadvantages)
3. Shorr (advantages and disadvantages)
4. Modified Papanicolaou techniques (advantages and disadvantages)
5. Supravital techniques (advantages and disadvantages)
6. Karyologic technique (advantages and disadvantages)
7. Phasemicroscopy on unfixed material (advantages and disadvantages)

C. EFFECTS OF ADMINISTERED ESTROGENS (EXOGENOUS ESTROGENS ONLY)

1. Metabolism of administered estrogens
2. Methods of determining the effect of administered estrogens other than exfoliative cytology

3. Do administered estrogens stimulate growth and maturation of the epithelium directly or indirectly through stimulation of the nerve fibers?
4. Cytological criteria of estrogen effect
5. Histological criteria of estrogen effect (epithelium and connective tissue)
6. Cytochemistry of cytoplasmic granules
7. Cytoplasmic granules and estrogen effect
8. Cytolysis and long-term administration of estrogens
9. Does one need to gradually increase the dosage of administered estrogens in patients under long-term estrogenic therapy in order to maintain high proliferation?
10. Can one induce a consistent intermediate type of proliferation by administering low dosages of estrogens?
11. Is there a condition known or is there a time period known in which the vaginal epithelium does not respond with marked proliferation to administered estrogens?
12. Can the karyopyknotic index be influenced by factors other than hormonal? If so, what are they?
13. Can the karyopyknotic index be influenced by hormones other than estrogens? If so, which hormones and to what extent.
14. Oral and buccal threshold dosages of administered estrogens.
15. Parenteral threshold dosages of administered estrogens.
16. Local threshold dosages of administered estrogens.
17. Why does an induced proliferation of an initially atrophic epithelium often persist considerably longer than the possible duration of the actual effect of the administered estrogen?
18. What are the relative dosages of androgens plus estrogens which suppress the occurrence of the highly proliferated cell type?
19. What are the relative dosages of progestogens plus estrogens which suppress the occurrence of the highly proliferated cell type.

* * *

VOLUME II 1958 NUMBER 3

The Written Symposion of this issue will be devoted to the discussion of ENDOMETRIAL CYTOLOGY.

The points of discussion will deal with normal, functional and abnormal cytology of the endometrium as well as techniques for obtaining cytological material in this particular field of exfoliative cytology.

Deadlines for this Issue

1. Members of the Academy who wish to be a main speaker or suggest guest speakers on any of the points listed below, must inform the Editorial Office not later than July 1, 1958 about their intention to actively participate.
2. The Main Papers must REACH the Editorial Office not later than September 1, 1958.
3. The Discussions must REACH the Editorial Office not later than November 1, 1958.
4. The Closing Remarks of the main speakers must REACH the Editorial Office not later than December 15, 1958.
5. The points of discussion are listed as proposed at this time. The Members of the Academy may have further points added to this list by writing to the Editorial Office not later than May 1, 1958. Members of the Academy who add additional points of discussion to the following list must, however, either agree to be the Main Speaker on this particular point or submit to the Editorial Office the name of an individual who who would agree to participate.

Preliminary list of Points of Discussion:

A. CANCER CYTOLOGY

1. Histomorphology of endometrial carcinoma
2. Histochemistry of endometrial carcinoma
3. Cytomorphology of normal endometrium
4. Cytochemistry of normal and abnormal endometrium
5. Phasemicroscopy on Endometrial Cells
6. Ultraviolet Microscopy on Endometrial Cells
7. Electron Microscopy on Endometrial Cells
8. Differentiation of Endocervical and Endometrial Cells
9. Histiocytes and Endometrial Cytology
10. Cytometry on normal and abnormal endometrial cells
11. Cytology of Endometritis
12. Cytology of Endometritis tuberculosa
13. Cytology of Endometrial Polyps
14. Cytology of Endometrial Adenocarcinomas
15. Cytology of Endometrial Adenoacanthomas
16. Cytology of the Irradiated Uterine Cavity
17. Cytology of Carcinoma in situ of the Endometrium
18. Cytology of Sarcoma and Chorionepithelioma

B. TECHNIQUES FOR ENDOMETRIAL CYTOLOGY

1. Endometrial Aspiration Technique (advantages and disadvantages)
2. Endometrial Brush Technique (advantages and disadvantages)
3. Cervical Pessary Collection Technique (advantages and disadvantages)
4. Vaginal Smears for detection of Endometrial Carcinoma (Diagnostic Accuracy)
5. Cervical Smears for detection of Endometrial Carcinoma (Diagnostic Accuracy)
6. Endocervical Smears for detection of Endometrial Carcinoma (Diagnostic Accuracy)
7. Are Degenerative Cell Changes in Endometrial Cells due to inadequate preparation techniques?

C. HORMONAL CYTOLOGY

1. Endometrial Cytology during the Follicular Phase of the Cycle
2. Endometrial Cytology during the Luteal Phase of the Cycle
3. Endometrial Cytology during and after the Menopause
4. Endometrial Cytology in Endometrial Hyperplasia
5. Endometrial Cytology Post Partum
6. Cytometry in Hormonal Evaluation
7. Diagnostic Accuracy of Hormonal Evaluation by Means of Endometrial Cytology
8. Correlative Studies of Endometrial Cytology, Endometrial Histology and Vaginal Cytology
9. Hormonal Evaluation of Patient with Endometrial Carcinoma (Vaginal Cytology and other hormonal evaluation techniques).

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VOLUME III 1959 NUMBER 1

The Written Symposion of this issue will be devoted to the discussion of CYTOLOGY DURING PREGNANCY.

The points of discussion will cover normal and disturbed pregnancy and carcinoma of the cervix during pregnancy.

Deadlines for this Issue

1. Members of the Academy who wish to be a Main Speaker on any of the points of discussion listed below, or suggest Guest Speakers must inform the Editorial Office not later than October 1, 1958 about their desire to actively participate.
2. The Main Papers must REACH the Editorial Office not later than December 1, 1958.
3. The Discussions must REACH the Editorial Office not later than February 1, 1959.
4. The Closing Remarks of the Main Speakers must REACH the Editorial Office not later than April 1, 1959.
5. A tentative list of proposed subjects is outlined below. The Members of the Academy may propose additional points for the list by writing to the Editorial Office not later than July 1, 1958. Members of the Academy who suggest additional points of discussion, however, must either agree to be the Main Speaker on this particular new point or submit the name of a willing contributor.

Preliminary List of Points of Discussion:

A. CANCER CYTOLOGY

1. The Squamous-Columnar Junctions during Pregnancy
2. Incidence of Cervical Carcinoma during Pregnancy
3. Prognosis of Cervical Carcinoma during Pregnancy as Compared with the Prognosis of Cervical Carcinoma in Non-Pregnant Women
4. Histomorphology of Cervical Carcinoma during Pregnancy
5. Histochemistry of Cervical Carcinoma during Pregnancy
6. Cytochemistry of Cells during Pregnancy (normal and abnormal)
7. Phasemicroscopy
8. UV Microscopy
9. Electron microscopy
10. Localization of Cervical Carcinoma during Pregnancy as Compared with Localization of Cervical Carcinoma in Non-Pregnant Women
11. Colposcopy during Pregnancy
12. Colpomicroscopy during Pregnancy
13. Cytology of Infections during Pregnancy
14. Cytology of Dysplasia during Pregnancy
15. Cytology of Carcinoma in situ during Pregnancy
16. Cytology of Invasive Carcinoma during Pregnancy
17. Clinical Viewpoints, with Special Consideration to Therapy and Time of Therapy for Lesions of the Uterine Cervix during Pregnancy
18. Experiences in the Cytological Detection of Cervical Carcinoma in Multiparas, as Compared to Primiparas
19. Diagnostic Accuracy of Colposcopy as Compared to Cytology in the Detection of Cervical Carcinoma during Pregnancy
20. Should all pregnant women be screened for carcinoma?

B. HORMONAL CYTOLOGY

1. Normal cytology during Pregnancy
2. Incidence of Cytolysis in smears during Pregnancy
3. Vaginal Flora of Pregnant Women as Compared to that of non-pregnant women
4. Vaginal Cytology as Prognostic Method in Pregnancy Disorders
5. Effect of Administered Estrogens on the Vaginal Epithelium in Pregnancy
6. Effect of Administered Progestogens on the Vaginal Epithelium in Pregnancy
7. Vaginal Cytology in the Diabetic Patient during Pregnancy
8. Vaginal Cytology Shortly Prior to Term
9. Vaginal Cytology After Rupture of Fetal Membranes
10. Vaginal Cytology Post Partum
11. Vaginal Cytology during Lactation Period
12. Vaginal Cytology in Abortion (excluding habitual aborters)
13. Vaginal Cytology in Habitual Aborters
14. Vaginal Cytology in Ectopic Pregnancy
15. Diagnosis of Pregnancy by Means of Cytology

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

ABSTRACTS

This portion of ACTA CYTOLOGICA includes abstracts (approximately 150–300 word each) of papers, either recently published or accepted for publication. Authors are invited to submit their own abstracts. Authors are requested to forward to the Editorial Office a complete manuscript or reprint of the original paper together with their abstract. All figures should be included.

The Editorial Office maintains a *free Literature Service* for distribution of available papers to cytologists. Authors are requested to send a minimum of 10 reprints, if possible, 150 copies of published papers to the Editorial Office. The Literature Service will make photostatic reproductions of papers which are unobtainable whenever possible.

RÉSUMÉS

Cette rubrique des ACTA CYTOLOGICA contient des résumés (d'environ 150 à 300 mots) de publications qui ont été récemment publiées ou acceptées pour la publication. Les auteurs sont priés de présenter leurs résumés *en anglais*. Les auteurs sont invités à faire parvenir au bureau de rédaction, en même temps que leur résumé, un manuscrit complet comprenant toutes les illustrations ou un tiré-à-part du travail original.

Le bureau de rédaction entretient *un service gratuit d'information littéraire* pour la distribution aux cytologistes de toute publication disponible. Les auteurs sont priés d'adresser au bureau de rédaction un minimum de 10, si possible 150 copies ou tirés-à-part de travaux publiés. Le service de documentation fera dans la mesure du possible des photocopies des publications épuisées.

ZUSAMMENFASSENDE BERICHTE AUS DER ZYTOLOGISCHEN LITERATUR

Dieser Teil der ACTA CYTOLOGICA beinhaltet zusammenfassende Berichte (von etwa 150 bis 300 Worten) von wissenschaftlichen Veröffentlichungen, die entweder schon publiziert oder zur Publikation angenommen worden sind. Autoren sind hiermit eingeladen, Zusammenfassungen ihrer Arbeiten (*in englischer Sprache*) an die Schriftleitung zu senden. Die Autoren sind gebeten, der Schriftleitung das vollständige Manuskript mit allen Abbildungen oder den Sonderdruck der Arbeit einzureichen.

Die Schriftleitung unterhält einen *kostenlosen Literatur-Dienst* zur Verteilung von wissenschaftlichen Arbeiten. Autoren sind gebeten, der Schriftleitung mindestens 10, möglichst aber 150 Kopien von Sonderdrucken ihrer Arbeiten einzureichen. Der Literatur-Dienst steht auch nach Möglichkeit zur Herstellung von Lichtkopien von schwer zugänglichen Arbeiten zur Verfügung.

RESUMENES

Esta parte de ACTA CYTOLOGICA incluye resúmenes (aproximadamente de 150–300 palabras cada uno) de los trabajos, publicados recientemente, o aceptados para su publicación. Los autores deberán enviar sus resumenes *en inglés*. Se requiere a los autores para que envien a la Oficina Editorial, junto con su resumen, un manuscrito completo o separata del trabajo original. Deberán incluirse todas las figuras.

La Oficina Editorial mantiene *un Servicio de Literatura, gratuito*, para la distribución de trabajos disponibles. Se ruega a los autores que envien a la Oficina Editorial un minimo de 10 copias de sus trabajos publicados y, de ser posible, 150 copias. El Servicio de Literatura hará, siempre que ello sea posible, reproducciones fotostáticas de los trabajos que los autores no puedan obtener.

HORMONAL CYTOLOGY

THE ANDROGENIC SMEARS (DIE ANDROGENEN ABSTRICHBILDER)

J. PAUL PUNDEL - Archiv fuer Gynaekologie 188: 577-588, 1957

This is a critical review of nearly all published papers on the action of androgens on the vaginal epithelium of females before and after the menopause. The findings of the different authors are compared and critically discussed. In general, there is now uniformity in their conclusions. Some of the differences or controversies can probably be adequately explained on the basis of different staining techniques, inadequacy of material, or difference in terminology, especially with reference to the definition of superficial cells and karyopyknosis. Numerous references have been supplied. (Author's abstract.)

VAGINAL CYTOLOGY AFTER OOPHORECTOMY AND ADRENALECTOMY (ETUDE DE LA CYTOLOGIE VAGINALE APRES OVARIECTOMIE ET SURRENALECTOMIE BILATERALES)

CLAUDE GOMPEL - Presse Medicale Paris 65: 538-540, 1957

A study of the vaginal exfoliative cytology, following bilateral oophorectomy and adrenalectomy, has demonstrated the gradual removal of the estrogenic activity. There was cytologic evidence of androgenic stimulation, after a prolonged interval, between the first and the second adrenalectomy. The disappearance, at times very slow, of this stimulation might be due either to the presence of stored estrogens in the tissues or to the presence of accessory adrenals, the hormonal activity of which would finally tend to disappear.

It is not possible to put forward a corresponding histological picture showing the resumption of growth activity in the tumor or the appearance of further hormonal activity. (Author's abstract.)

COLPOCYTOLOGY IN POSTMENOPAUSAL PATIENTS (HALLAZGOS COLPOCITOLOGICO EN LA POSTMENOPAUSIA)

L. MONTALVO RUIZ - Acta Ginecologica, Date of acceptance: September 1957

This is a study of 50 menopausal women, 40 of them were selected and 10 eliminated as they had been treated with estrogens. In 40% of these women, more or less marked estorgenic activity was in evidence cytologically. One of them was 83 years old and for 32 years menopausal, without any functioning tumour. Estrogen activity depends initially upon the functioning ovary but from the fifth menopausal year onwards it is rather difficult to agree that atrophic ovaries still produce hormones. We believe with Botella that the suprarenal is the source of these estrogens, as it is shown by the findings in the smears of castrated women. (Author's abstract.)

THE DIAGNOSIS OF OVULATION BY ENDOMETRIAL CYTOLOGY

CLARICE AMARAL FERREIRA - International J. of Fert. 2: 141-154, 1957 and An. Bri. Gin. 43: No. 3, 1957.

A study of endometrial smears from 110 patients referred for Fertility Consultation is presented. In 36 of these cases, glycogen studies were done. The stromal and glandular cell aspects of the different stages of the normal cycle are presented. The cellular morphology in the disturbed and anovulatory cycle is also discussed. The author states in conclusion that the cytological study of endometrium, like the histologic study, permits recognition of its proliferative and secretory phases, thus facilitating a cytohormonal diagnosis. In endometrial or ovulatory disturbances the cytologic pattern coincides with the histologic one. Whenever histologic study is impossible, cytologic study may prove a satisfactory substitute. It is possible to make a cytohormonal diagnosis, many times in the same cycle, without discomfort to the patient. Thus one can see the evolution of the endometrial picture.

Staining for glycogen helps in the recognition of the different phases of the endometrial cycle and permits recognition of sterility due to lack of endometrial glycogen. (Author's abstract.)

(The above two papers by Ferreira are important contributions for those of us concerned with endocrinological studies. One often observes various histological degrees of either proliferation or secretion if one makes in the same patient three minute endometrial biopsies at different places in the uterine cavity. Cytological findings may offer more uniform results in endocrinological studies than those resulting from minute biopsy samples of the endometrium. The papers by Ferreira should be read in the original. - Editor).

CLIMACTERIC AMENORRHEA. A CYTOHORMONAL TEST FOR DIFFERENTIAL DIAGNOSIS

GEORGE L. WIED - Obst. and Gynecology 9: 646-649, 1957

A cytohormonal test which may be useful in the differential diagnosis of climacteric amenorrhea (estrogen deficiency) and pregnancy is described. The test necessitates the oral administration of estrogens

over a period of four days and two cytological examinations of the vaginal smear. It is emphasized that the preparation of the initial smears and their proper evaluation is essential to the reliability of the test. Performed accurately the test has been shown to be reliable as early as the second or third week after conception. Factors such as infection, Trichomonas infestation, and bacterial cytolysis, which may limit the value of this test, are described. (Author's abstract.)

PROGESTATIONAL SUBSTANCES TESTED ON THE HIGHLY PROLIFERATED VAGINAL EPITHELIUM OF SURGICAL CASTRATES

GEORGE L. WIED, JOSE R. DEL SOL and ALICE M. DARGAN - Am. J. Obst. & Gyn., Date of acceptance: May, 1957.

The effects of 17α-hydroxyprogesterone caproate, chemically pure progesterone and anhydrohydroxyprogesterone on the estrogen-stimulated, highly proliferated vaginal cells and endometrium of six surgical castrates were evaluated and compared. The object was to determine whether or not cytological changes could be induced in the highly proliferated vaginal epithelium which would compare with those observed during the luteal phase of the normal menstrual cycle, and, if so, which dosage would be necessary to induce these changes. It has been found that dosages of progestational agents sufficient to induce complete secretory changes in the endometrium indistinguishable from those in the late secretory phase of the normal menstrual cycle also induce so-called luteal changes in the vaginal epithelium. (Authors' abstract.)

ANDROGENIC SUBSTANCES TESTED ON THE HIGHLY PROLIFERATED VAGINAL EPITHELIUM OF SURGICAL CASTRATES

GEORGE L. WIED, JOSE R. DEL SOL, and ALICE M. DARGAN - Am. J. Obst. & Gyn., Date of acceptance: September, 1957

The degree and duration of the androgenic activity of two esters of testosterone, testosterone propionate and testosterone enanthate, were evaluated and compared by means of a "regression test" on the estrogen-stimulated, highly proliferated vaginal epithelium of surgical castrates. It is shown that this "regression test" as well as the previously reported "proliferation test" on the atrophic vaginal epithelium offers a method of assessing androgenic activity. (Authors' abstract.)

CYTOLOGY IN INFLAMMATORY REACTIONS

EXPERIENCES WITH ALBOTHYL IN THE GYNECOLOGICAL PRACTICE (ERFAHRUNGEN MIT ALBOTHYL IN DER GYNAEKOLOGISCHEN PRAXIS)

PETER STOLL and HANS POLLMANN - Muenchener Med. Wschr., 99: 1719-1726, 1957

The authors treated patients with various gynecological conditions, such as inflammatory reactions on the cervix uteri, ectopies, vaginitis, Trichomonas infestation and non-specific inflammatory lesions produced by pessaries, with a new substance Dioxy-dimethyl-diphenylmethan-disulfonic acid (Albothyl). The authors controlled their therapy by means of colposcopy, cytology (phase-contrast microscopy on fresh specimens and routine Papanicolaou staining), and in some cases by means of histology. The authors conclude that the control of therapeutic procedures by means of these above techniques offers an excellent way to assess therapy by correlating the subjective findings with colposcopy and cytology. (Authors' abstract.)

CYTOLOGICAL STUDY IN CHRONIC CERVICITIS

CLARICE AMARAL FERREIRA and GEORG SCHNEIDER - An. Br. Gin., Date of acceptance: August, 1957.

The authors report a statistical study of the cytologic pictures in a series of 438 cases with chronic cervicitis.

In a group of 100 patients with chronic cervicitis submitted to colposcopic, cytological and clinical studies, the authors report their findings in 70. They agree, that in chronic cervicitis, cytology plays a multiple role, not only as a diagnostic aid, but for the control of treatment. In addition, it is a screening method for detection of malignant lesions and an aid in cancer prevention. (Authors' abstract.)

THE INTERPRETATION OF INFLAMMATORY REACTIONS IN THE VAGINA, CERVIX, AND ENDOCERVIX, BY MEANS OF CYTOLOGIC SMEARS

GEORGE L. WIED - Am. J. Clinical Pathology 28: 233-242, October, 1957

The potentialities of the cytological smear examination with reference to the localization of inflammatory reactions in the vagina, cervix or endocervix, and the tentative classification of these inflammatory states into mild, marked, acute and chronic reactions, are discussed. Three smears (vaginal, cervical and endocervical) are provided on the same glass slide. The differentiation results then from the comparative evaluation of the leukocytes present in the three smears, their stage of preservation, the absence or presence of histiocytes, and finally the presence or absence of so-called inflammatory epithelial cell changes. (Author's abstract.)

CANCER CYTOLOGY

KARYOLOGICAL PROPERTIES OF CANCER CELLS. A STUDY ON CARCINOMAS OF THE UTERINE CERVIX (KARYOLOGISCHE MERKMALE DER KREBSZELLE, AN COLLUMCARCONOMEN UNTERSUCHT)

PETER STOLL and GUENTHER ECKERLE - Archiv fuer Geschwulstforschung 11: 89-101, 1957

The authors examined exfoliated cells from cervical carcinomas after fixation with alcohol and acetic acid, and staining with orcein and carmine, respectively.

The hyperchromatic nuclei of carcinoma cells are subdivided into three groups. The nuclei within the three groups vary with the type of nuclear mitosis, the size, the chromatin distribution, the form of the nucleoli and the nuclear-nucleolar ratio. In addition to that the authors report small nuclei, the exact derivation of which is questionable. Carcinomas of the mature cell type exfoliate sometimes individual giant cells.

The hypochromatic nuclei of the carcinoma cells show generally an increase in volume as compared with the hyperchromatic nuclei. The hypochromatic nuclei exhibit again three types of regressive changes.

The authors have investigated the accuracy of the karyological diagnosis of cancer as introduced by L. CUSMANO. They report his findings as reproduceable and accurate. The practical use of the karyological technique for routine cancer detection does not offer, however, advantages over the classical PAPANICOLAOU technique. (Authors' abstract.)

(The paper critically examines a problem which often occurs especially in the Italian literature. It is difficult to see how anyone could utilize the karyological technique on a practical basis without loss of some important potentialities of exfoliative cytology. This important paper should be read in the original. - Editor).

EFFECT OF HORSE ANTI-HUMAN CANCER SERUM ON MALIGNANT AND NORMAL HUMAN CELLS

BERTIL BJORKLUND, JOHN B. GRAHAM and RUTH M. GRAHAM - Int. Archives Allergy and Applied Immunology 10: 56-63, 1957

1. Anti-tumor serum was produced in a horse by repeated injection of pooled human cancer tissue. This serum was treated in order to neutralize the antibodies to normal human plasma constituents. 2. The treated serum was tested in tissue culture according to Pulvertaft's method for biological effects on freshly-isolated human tumour cells, as well as on normal cells. 3. As a whole, the experiments showed that cervical cancer cells freshly-isolated from patients are more sensitive to antibodies to pooled human cancer tissue than freshly-isolated normal cells from the vagina and endometrium. (Authors' abstract.)

CONSERVATIVE SURGERY FOR EARLY CERVICAL CANCER IN YOUNG WOMEN

J. E. AYRE, A. CASTILLO, W. S. ROGERS and R. JACK - Obstetrics & Gynecology November, 1957

Radical surgery or extensive radiation therapy is no longer necessary to cure early cervical cancer detected cytologically in the preclinical or preinvasive stage. The widespread use of cytologic scrapings, using a cervical spatula, identifies the cancer while in an asymptomatic, preclinical stage; while the lesion is confined to the region of the squamocolumnar junction. Precise surgical excision is made possible by the Ring-Biopsy, followed by electroconization, removing a cone of tissue which includes the entire squamocolumnar junctional circle. Surgical bleeding is prevented by prior infiltration of the tissues with 1% novocaine with adrenalin. It must be proven by histological examination of quadrant biopsies from the ring of tissue and serial sections through the zone of the neoplasm that no definite infiltration has taken place. Proof of adequacy of surgical extirpation of neoplasm depends upon repeated negative cervical cell scrapings extending over a two-year period. When these criteria have been complied with, the pre-clinical malignancy may be considered completely eradicated and pregnancy may be permitted. This precise method of dealing with early cancer in young women represents a significant forward step in the treatment of early malignant disease, preserving the reproductive function and avoiding the crippling effects of radical surgery or radiation. (Authors' abstract.)

(The conservative therapy of carcinoma in situ is favourably described by many authors, such as K. H. Bruntsch [Med. Klinik 50: 1741, 1955], T. W. Huey, H. Lee Large and P. Kimmelstiel [Am. J. Obst. & Gyn. 68: 761, 1954], J. P. A. Latour, L. B. Brown and L. A. Turnbull [Am. J. Obst. & Gyn. 74: 354, 1957]. Other authors, such as J. B. Gusberg and D. B. Moore [Obst. & Gyn. 2: 1, 1953] are not in favour of this type of conservative treatment, and J. M. Blumberg and W. B. Ober [Am. J. Obst. & Gyn. 66: 421, 1953] report recurrence of carcinoma in situ in the vaginal vault after therapy by hysterectomy. P. A. Younge [New York J. Med. 50: 21, 1950] found that such conservative treatment was a failure in 10 out of 16 cases.

Academically speaking, a non-invasive carcinoma [carcinoma in situ] should be cured after local removal of the entire lesion. The only practical considerations are: (1) did one remove the entire lesion? and (2) is there definitely no evidence of invasion in the removed specimen? Exfoliative cytology might be of great help in answering the first question. The second question can only be answered by serial sections of the entire cone with demonstration of all limitations of the lesions against the normal

tissue. There are few pathology laboratories in any country capable of providing their clinicians with a report on the examination of hundreds or thousands of histological sections from each removed cervical cone; and this is the only way of proving that there is definitely no invasion present. The standards of a highly specialized research institution such as that of the above authors are not found in every hospital. Removal of a cervical cone and subsequent examination with only 8 or 16 histological sections may in some cases yield poor results as early invasive lesions might be missed by technically inadequate histological examinations. - Editor.)

THE CYTOLOGIC DIAGNOSIS OF CANCER

OLLE KJELLGREN - Scandinavian Textbook of Tumour Diagnosis and Radiotherapy (in Swedish), Date of acceptance: October, 1957

A review of the possibilities of cancer diagnosis by means of exfoliative cytology in the fields of gynecology, gastro-enterology, urology, pulmonary disease and in diseases of the mammary gland is presented. (Author's abstract.)

EARLY DETECTION OF GYNECOLOGICAL CARCINOMAS (FRUEHDIAGNOSE GYNAEKOLOGISCHER CARCINOME)

PETER STOLL - Die Therapiewoche 7: 231, 1957

The successful treatment of carcinomas depends upon the following factors: time of diagnosis, onset of adequate therapy, localization of the carcinoma, type of carcinoma, host defense reaction, post therapeutic treatment. The early detection seems to be one of the most important points among the above listed factors. The time of diagnosis depends again on the following factors: education of the general public, cooperation of the patient, localization and symptoms of the carcinoma, and training of the medical profession in techniques of early cancer detection.

The author describes various diagnostic techniques which vary with the site of the tumor. As far as the cervical carcinomas are concerned cytology and colposcopy should be added to the routine gynecological examination. The definitive diagnosis will be the histological one. The diagnostic accuracy of the techniques are compared. It is suggested that cancer detection may be done in the office of the practicing physician by introducing consultants in colposcopy and centralized laboratories for cytology. (Author's abstract.)

THE CYTOLOGICAL DIFFERENTIATION OF CARCINOMA IN SITU AND INVASIVE CERVICAL CARCINOMA (DIFFERENCIAS CITOLOGICAS ENTRE EL CANCER IN SITU Y EL CANCER INFILTRANTE DEL CERVIX)

JORGE CAMPOS R. DE C. and OSCAR MISAD - Ginec. y Obst. 2: 81-87, 1957

1900 malignant cells from cytological smears of 50 cases of carcinoma in situ and 520 cells from smears of 12 cases of invasive cervical carcinoma, all confirmed histologically, have been studied. Measurements of each cell and its nucleus have been made and the morphological characteristics of the nucleus and of the cytoplasm have been described. The authors conclude that there are no neoplastic cells pathognomonic of carcinoma in situ which would permit the diagnosis by cytology alone. However, the smear of carcinoma in situ is usually highly suggestive of and allows the pathologist, in most cases, to anticipate the possibility of such a diagnosis which must then be confirmed histologically in every case. (Authors' abstract.)

(These findings are very interesting in view of the current discussions as to whether or not one should attempt to anticipate the histological findings by means of cytology. I believe one should make full use of the potentialities of cytology which include the tentative diagnosis of the possible nature of the cervical lesion, provided that one has as critical an approach as the author of this article. - Editor.)

CYTOLOGICAL DIAGNOSIS IN PRECANCER, CANCER IN SITU AND CANCER OF THE UTERINE CERVIX (in Swedish)

OLLE KJELLGREN - Proc. Northern Assoc. Obst. & Gynec. 119, 1957

A short review of the cytological method and the results obtained in various neoplastic conditions of the uterine cervix. (Author's abstract.)

VAGINAL CYTOLOGY DURING RADIUM THERAPY FOR CERVICAL CARCINOMA (MODIFICACIONES DE LA CITOLOGIA VAGINAL EN ENFERMAS DE CARCINOMA DE CUELLO UTERINO DURANTE SU TRATAMIENTO CON RADIUM)

L. MONTALVO RUIZ and V. JIMENEZ TEBAR - Bol. de la Sociedad Ginecologica Espanola 7: 120, 1957

Since 1953 we have studied the alterations in the vaginal cytology of cancer patients who have been subjected to irradiation. The first specimen was taken before the application of radium; a second one seven days after having received 2300 mg. and a third one ten days after having completed 4600 mg. All the patients treated were observed every three months during the first year and every six months in the succeeding years up until the present date. In a series of 54 cases completely studied, we found 31 patients with a good cytologic reaction (according to Graham's criterion); 23 of these women are still alive without any recurrence; some of them for already more than four years.

There were 21 patients with a bad reaction, 8 or them are still alive and the other 13 are either dead or have a recurrence. As the number of patients studied is very small, we are not able to draw any conclusions. (Authors' abstract.)

CYTOLOGIC STUDIES OF NIPPLE DISCHARGE

OLLE KJELLGREN - Acta radiol. 46: 753, 1956

The frequency of bleeding from the nipple in cancer of the breast is stated to vary between 1 and 5 per cent. The frequency of mammary cancer in patients with such bleeding is reported to be 36 to 50 per cent. Growing intraductal papillomas as a source of bleeding from the nipple is given as 11 to 44 per cent.

Thirty-nine patients with nipple discharge were examined cytologically according to PAPANICOLAOU'S technique. The method and the findings are discussed. The cytologic examination seems to offer an additional diagnostic possibility in cases of discharge from the nipple. The results are encouraging and the method appears worth trying on a larger group of cases. (Author's abstract.)

THIS AND THAT

This portion of ACTA CYTOLOGICA is devoted to miscellaneous information.

INFORMATIONS DIVERSES

Cette rubrique des ACTA CYTOLOGICA est destinée à des informations diverses.

PERSÖNLICHE INFORMATIONEN

Dieser Teil der ACTA CYTOLOGICA befasst sich mit verschiedenen persönlichen Nachrichten.

ESTO Y AQUELLO

Esta parte de ACTA CYTOLOGICA está dedicada a información diversa.

J. ERNEST AYRE OF MIAMI, FLORIDA, U.S.A. was elected Honorary Member of the Brazilian Society of Cytology of which Professor Arnaldo de Moraes is president. Dr. Ayre visited the Dominican Republic and gave a lecture for the Medical faculty of the University of Santo Domingo entitled "The Importance of Early Cytological Diagnosis of Cancer."

JEAN BERGER OF BASEL, SWITZERLAND was in charge of the committee at the meeting of the German-speaking professors and professorial lecturers in obstetrics and gynecology, in Basel, Switzerland, October 15-17, 1957.

H. WERNER BOSCHANN OF WEST-BERLIN, GERMANY was the guest speaker at the meeting of the New York Academy of Sciences on "New Progestational Agents" at which Drs. Rakoff and Reifenstein were the Chairmen. Dr. Boschann later visited Drs. Rakoff, Ayre, Graham, Reagan and Wied. On his way back to Berlin, he spoke at the University of Madrid, Spain, on "Progestational Agents Tested on the Endometrium and Vaginal Epithelium" by invitation of Professor Botella Llusia.

JOSE BOTELLA LLUSIA OF MADRID, SPAIN participated in the meeting of the Gynecologists of Spain, in Malaga, Spain, December 10-13, 1957.

JEAN DE BRUX OF PARIS, FRANCE and PROFESSOR FUNK-BRENTANO were in charge of an extensive course in exfoliative cytology at the University of Paris, France during the month of December, 1957. Drs. Funk-Brentano and De Brux are also preparing the foundation for a European Society of Cytology during the year 1958.

JORGE CAMPOS R. OF LIMA, PERU was elected secretary of the First National Conference of Pathology of Peru and participated in the Third National Cancer Congress in Chile in November, 1957.

LOWELL T. COGGESHALL OF CHICAGO, ILLINOIS, U.S.A., Dean of the Division of the Biological Sciences at the University of Chicago, was elected President of the American Cancer Society, November 1, 1957. Dr. Coggeshall was elected President of the Association of American Medical Colleges, October 23, 1957.

W. KENNETH CUYLER OF DURHAM, NORTH CAROLINA, U.S.A. participated in the panel conference on carcinoma in situ during the meeting of the Inter-Society Cytology Council in Augusta, Georgia November 14-16, 1957. Dr. Cuyler was elected Member of the Executive Council of this organization.

EMERSON DAY OF NEW YORK, NEW YORK, U.S.A. has been elected President of the Inter-Society Cytology Council at the meeting of this organization in Augusta, Georgia in November, 1957. The next meeting of this organization will be held in New York City's Statler Hotel probably in November, 1958.

WILLIAM J. DIECKMANN OF CHICAGO, ILLINOIS, U.S.A., Editor of the American Journal of Obstetrics and Gynecology, and former Chairman of the Department of Obstetrics and Gynecology of the University of Chicago, died in Chicago, August 15, 1957.

RUTH M. GRAHAM OF BUFFALO, NEW YORK, U.S.A. will visit Great Britain in March, 1958, and will attend the International Cancer Congress in London, in July.

OLAF T. MESSELT OF OSLO, NORWAY plans to visit the United States of America during the months of January and February, 1958.

JUNJI MIZUNO OF NAGOYA, JAPAN will participate in the Second World Congress of the International Federation of Gynecology and Obstetrics in Montreal, Canada in June 1958. On this occasion he plans to visit with Drs. Graham of Buffalo, New York and Wied of Chicago, Illinois.

LUIS MONTALVO RUIZ OF MADRID, SPAIN participated in the meeting of the Gynecologists of Spain, in Malaga, Spain, December 10-13, 1957.

GEORGE N. PAPANICOLAOU OF NEW YORK, NEW YORK, U.S.A. who was the President of the Inter-Society Cytology Council for the past year, presided over the meeting of this organization in Augusta, Georgia, November 14-16, 1957. Dr. Papanicolaou presented a paper on tissue culture of endometrial cells, at that meeting.

J. PAUL PUNDEL OF LUXEMBOURG, LUXEMBOURG participated as guest lecturer in the cytology course at the University of Paris, in December, 1957.

A. E. RAKOFF OF PHILADELPHIA, PENNSYLVANIA, U.S.A. was the Chairman of the meeting of the New York Academy of Sciences on "New Progestational Agents" in October, 1957. Dr. Rakoff who is a Vice-President of the Inter-Society Cytology Council was the Chairman of several scientific sessions at the meeting of this organization and presented together with Dr. Warren R. Lang a paper on colposcopy in pregnant women.

JAMES W. REAGAN OF CLEVELAND, OHIO, U.S.A. conducted a cytology panel conference during the meeting of the College of American Pathologists in New Orleans, in October, 1957.

EDMUND SCHUELLER OF VIENNA, AUSTRIA participated in the meeting of the German-speaking professors and professorial lecturers of obstetrics and gynecology in Basel, Switzerland, October 15-17, 1957.

JOSE R. DEL SOL OF MADRID, SPAIN participated in the meeting of the Gynecologists of Spain, in Malaga, Spain, December 10-13, 1957.

PETER STOLL OF HEIDELBERG, GERMANY and PROFESSOR H. RUNGE are preparing the foundation for a new European Society for Cytology which is scheduled to be founded during the year 1958. Professor H. Runge will most probably be the Chairman of the German Section and Dr. Peter Stoll the Secretary. Dr. Stoll participated in the meeting of the German-speaking professors and professorial lecturers of obstetrics and gynecology in Basel, Switzerland, October 15-17, 1957.

JOHN J. SULLIVAN OF AUCKLAND, NEW ZEALAND is on a world tour at this time visiting various cytology centers. After his stay with Drs. Graham of Buffalo, Koss of New York City, Reagan of Cleveland and Wied of Chicago, he plans to visit with Drs. Wachtel of London, Montalvo and Del Sol of Madrid, De Brux of Paris, Zinser of Cologne, Stoll of Heidelberg, Boschann of Berlin, and Pundel of Luxembourg. Dr. Sullivan plans to return to New Zealand via India and will visit Dr. Peters of Bombay. Dr. Sullivan is accompanied by Mrs. Sullivan.

GUILLERMO TERZANO OF BUENOS AIRES, ARGENTINA will attend the International Cancer Congress in London, in July, 1958.

ERICA WACHTEL OF LONDON, ENGLAND participated as guest lecturer in the Cytology Course at the University of Paris during the month of December, 1957.

GEORGE L. WIED OF CHICAGO, ILLINOIS, U.S.A. participated together with Dr. M. Edward Davis in the meeting of the New York Academy of Sciences on "New Progestational Agents," in October, 1957. He also participated in the meeting of the Academy of General Practice in Springfield, Illinois, in October, 1957. In addition he participated in the panel discussion on carcinoma in situ at the meeting of the Inter-Society Cytology Council in Augusta, Georgia, and presented a paper on "Identification of Local Inflammatory Reactions by Means of Cytology." He was elected Corresponding Member of the Brazilian Cytology Society.

HANS KLAUS ZINSER OF COLOGNE, WEST-GERMANY opened a new cancer detection clinic in his hospital, the Evangelical Hospital of Cologne-Lindenthal. Dr. Zinser participated in the meeting of the German-speaking professors of obstetrics and gynecology in Basel, Switzerland, October 15-17, 1957.

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The Editor wishes to apologize if some of the Members' activities were not recorded and if some of the Members were not mentioned. This is due to difficulties in compiling the data, and the lack of information received in the Editorial Office. The Members are invited again to supply the Editorial Office with all relevant information for this column.

WANTED OR AVAILABLE

It is the purpose of this column to promote international exchange of cytologists and cytotechnicians, to inform them of open permanent positions, and to inform employers of available cytology personnel. The ACTA CYTOLOGICA offers this service free of charge to Members of the Academy and also to Non-Members. Persons interested in obtaining permanent positions as cytologists or cytotechnicians or in obtaining temporary fellowships in cytology (teaching, exchange, or training fellowships), and individuals or institutions offering such positions or openings are invited to write to: the ACTA CYTOLOGICA (5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.) giving full information. Information supplied will be held strictly confidential.

While information received is subject to editing so that it conforms to the style of the ACTA CYTOLOGICA, the ACTA CYTOLOGICA and the International Academy of Gynecological Cytology cannot and do not assume responsibility for statements made by contributors.

CYTOLOGISTS AND CYTOTECHNICIANS AVAILABLE

CYTOLOGIST-GYNECOLOGIST, Female, Age: 34, citizen of West-Germany, single, graduate of West-German Medical School (M.D. = Dr. med.).

Medical Training: Eligible for German Board of Obstetrics and Gynecology (Facharzt fuer Frauenheilkunde). Completed additional internship and full residency training in Obstetrics and Gynecology in the United States of America.
Cytology Training: (1) in West-German Medical School, Department of Obstetrics and Gynecology and (2) one and a half years full-time cytology fellowship in a training center in the United States (approved by the American Cancer Society).
Wanted: Position as gynecologist in charge of Cytology Laboratory (with clinical work if desirable) in the United States of America, Canada, or West-Berlin, Germany.
Code No.: OMD 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago, Illinois, U.S.A.

CYTOLOGIST-PATHOLOGIST, Male, Age: 36, single, graduate from Italian University (M.D.), citizen of Italy.

Medical Training: Pathologist with six years experience after completion of residency.
Cytology Training: Self-trained.
Wanted: Training Fellowship in Cytology or Research Associate in Cytology in Austria, Germany, or Switzerland for 6 to 12 months.
Code No.: LLM 1/1/57 in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago, Illinois, U.S.A.

GYNECOLOGIST, Male, Age: 33, citizen of Denmark, married, graduate of Danish University (M.D.).

Medical Training: Completed internship and residency in Obstetrics and Gynecology, and 3-1/4 years staff member in University Hospital.
Cytology Training: None.
Wanted: Training Fellowship in Cytology in Great Britain or Central Europe.
Code No.: ANV 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago, Illinois, U.S.A.

CYTOTECHNICIAN, Female, Age: 25, single, citizen of the United States of America, University Graduate.

Cytology Experience: Presently Chief-Cytotechnician in cytology laboratory in a Medical School in the United States of America. This particular laboratory is a training laboratory approved by the American Cancer Society. Co-author of several scientific publications on cytology.
Wanted: Teaching fellowship for 3-4 months to organize, set up, or modernize cytology laboratories. Would consider India, Australia, New Zealand, Ireland, or Africa. Will return to present position in United States upon completion of fellowship.
Code No.: UG 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago, Illinois, U.S.A.

CYTOTECHNICIAN, Female, Age: 32, citizen of West-Germany, single, registered medical technician.

Cytology Experience: Chief-Cytotechnician 7 years in cytology laboratory of a University De-

partment of Obstetrics and Gynecology in Germany. Experienced in cancer cytology, endocrinological cytology and hematology.

Wanted: Exchange fellowship for a period of several months with a cytology center in the United States of America, Brazil, or Argentina. Will return to present position upon completion of fellowship.

Code No.: RU 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago, Illinois, U.S.A.

CYTOLOGISTS AND CYTOTECHNICIANS WANTED

TRAINEES IN EXFOLIATIVE CYTOLOGY WANTED in Laboratory of Exfoliative Cytology in a Medical school in the United States of America. The prospective applicants must be either citizens of the United States of America, or have taken out citizenship papers (immigrant); must be high school graduate or have equivalent credits with some training in biological sciences; no physical or mental disabilities that would interfere with training or restrict services as a cytology technician after training. Trainees will be awarded a stipend for a period of six months at the rate of $225.00 per month.

Write to: ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago, Illinois, U.S.A., and refer to: U. S. Public Health Service Traineeship.

CYTOTECHNICIANS WANTED for the Laboratory of Exfoliative Cytology of the University of Chicago Clinics. The prospective applicants should be high school graduates with 18 semester hours of courses in biological sciences who want to make cytology his or her career or/and who has previous training in exfoliative cytology. The salary is adjusted according to the regulations of the University of Chicago Personnel Office.

Write to: George L. Wied, M.D., Chicago Lying-In Hospital, Chicago 37, Illinois, U.S.A.

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